EFFECTS OF FORCED ALKALINE DIURESIS ON SALICYLATE DISTRIBUTION AND ELIMINATION FOLLOWING OVERDOSAGE

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DECLARATION

I declare that this Thesis is of my own composition. Being a member of a research group I have made a contribution to the development of the High Performance Liquid Chromatography methods described. The clinical studies incorporated into this Thesis are entirely my own work. The help given by others is acknowledged on page 297 and all sources of information are indicated in the text.

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ABSTRACT

The effects of changes in urine flow rate and pH on the distribution and elimination of salicylates was compared in 12 healthy volunteers given therapeutic doses of aspirin and diflunisal and 50 patients with aspirin overdosage. Specific, sensitive and reproducible high performance liquid chromatographic methods were developed for the estimation of acetylsalicylic, salicylic and salicyluric acids and diflunisal in plasma and urine. Salicylate protein binding was investigated in-vitro and in-vivo and compared with albumin binding using an ultrafiltration technique.

Alkalinisation of the urine by oral administration of sodium bicarbonate in the healthy volunteers given 20 mg/kg aspirin orally resulted in a significant increase in the total body and renal clearances of salicylic acid but not salicyluric acid. Urinary recovery of acetylsalicylic and salicylic acids were markedly increased with alkaline diuresis whereas the salicyluric acid recovery was significantly decreased. There were significant positive correlations between the renal clearance of salicylic acid and urine pH or flow rate, but in a corresponding study with diflunisal, there were no such relationships. Forced alkaline diuresis would therefore be ineffective in diflunisal poisoning.

Adult patients with aspirin overdosage (plasma concentrations 400-800 μg/ml) were treated with one of four i.v. regimes of fluid and alkali (over 3 hr) to establish the relative importance of urine flow rate and pH on salicylate elimination. Sixteen patients received forced alkaline diuresis (FAD - 18.9 g $NAHCO_3$ in 6L isotonic fluid), 6 forced diuresis (FD - 6L isotonic fluid), 6 alkali (A - 18.9 g $NaHCO_3$ in /

in 1½L) and 6 FAD with 80 mg i.v. frusemide (FAD + F). No specific treatment was given to 16 control patients with mild salicylism (300-450 μg/ml).

Acetylsalicylic acid was rapidly hydrolysed to salicylic acid and was only detected when absorption was delayed (25 patients). All treatments produced similar initial rapid falls in plasma salicylic acid concentrations during the infusion period (0-4 hr), but urinary salicylate excretion was only increased with the alkalinisation regimes. Haemodilution and increased apparent volume of salicylate distribution probably contributed to this initial fall particularly in the non-alkalinisation regime. From 4-16 hours the mean plasma elimination half-lives of salicylic acid were 30, 31, 12, 14 and 9 hours in the control, FD, FAD, FAD + F and A groups respectively. Total body clearance, renal clearance and urinary recovery of salicylic acid were significantly higher with the alkalinisation regimes than with forced diuresis alone. The renal clearance of salicylic acid was a function of both urine flow and pH in all groups. There was a direct relation with the former but the influence of pH increased dramatically above 7.0 where it completely dominated the elimination kinetics of salicylic acid. The mean renal clearances of salicylic acid during the first 16 hours in the control, FD, FAD, FAD + F and A groups were 1.4, 4.4, 18, 13 and 24 ml/min respectively.

The binding of salicylic acid to plasma proteins was highly concentration-dependent and the mean unbound fraction rose from 14% at 50 μg/ml to 80% at 800 μg/ml, a finding of toxicological significance. Salicylic acid was bound, in-vitro, less to albumin than to equivalent concentrations of total plasma proteins.

Plasma concentrations of salicyluric acid were uniformly very

low /

low (< 10 μg/ml) and constant in all groups which reflected saturation of glycine conjugation of salicylic acid. The very high renal clearance of salicyluric acid was independent of urine flow rate or pH.

Fluid retention and weight gain were significantly greater and haematological and biochemical abnormalities resulting from haemodilution were more obvious in the forced diuresis and FAD groups. Administration of alkali alone was more effective than the other regimes including FAD for the treatment of acetylsalicylic acid poisoning.

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SECTION I

INTRODUCTION

SECTION I

Chapter 1. REVIEW OF PHARMACOLOGY AND TOXICOLOGY OF SALICYLATES

(a) History

Drugs have been used to treat diseases for thousands of years, traditionally their effectiveness was established by trial and error. Hippocrates some 2,400 years ago recommended leaves of willow tree in childbirth, Pliny made a paste from the ash of willow bark for removing corns and callosities in the first century (Gross and Greenberg, 1948; Baywaters, 1962; Avicenna, in the eleventh century, included willow bark as one of his medicinal drugs (Girdwood, 1976).

In the 18th century, the antipyretic properties of the bark of willow "salix alba vulgaris" were discovered in England by the Reverend Edward Stone, in an attempt to find a cheap substitute for expensive imported cinchona, a bark which contains quinine, and was used as antipyretic analgesic. He carried out a clinical trial of 50 patients with the "auge" each of whom received 20 grains of powdered willow bark extracted in a dram of water every 4 hours. According to Stone, the results were excellent (Bowman and Rand, 1980). The active ingredient in the willow bark was a bitter glycoside called salicin, first discovered by Leroux in 1827. On hydrolysis, salicin liberates glucose and salicylic alcohol (saligenin). Piria, in 1838, made salicylic acid from salicin. Six years later, salicylic acid was prepared from oil of gaultheria (oil of wintergreen) by Cahours (Martin, 1962; Flower, Moncada and Vane, 1980). In fact, the word "salicylate" is derived from the botanical name for the /

the willow family "salicaceae" (Bowman and Rand, 1980). Kolbe and Lautemann produced salicylic acid synthetically from phenol in 1860 and Kolbe devised a commercially practical process in 1874 (Sollman, 1936). Sodium salicylate was first used as an antipyretic by Buss in 1875, and in the following year its value in rheumatic fever was discovered by Stricker. Phenyl salicylate was introduced into medicine in 1866 by Nencki. Moore (1879) reviewed thousands of cases of various diseases treated with salicylic acid and its compounds. Acetylsalicylic acid was first synthesised in 1897 by Hoffman and introduced into the clinical medicine under the trade name of aspirin in 1899 by Dresser. The name aspirin comes from "Spirsäure" the German word for salicylic acid (Goodman and Gillman, 1956).

Many salicylic acid derivatives have been synthesised and introduced into medicine in this century. Diflunisal, one of the new salicylates discovered in 1971 has been used as an analgesic anti-inflammatory agent in clinical practice since 1977 (Hannah, Ruyle, Jones, Matzuk, Kelly, Witzel, Holtz, Houser, Shen and Sarett, 1977).

In 1798, Longmore described methyl salicylate poisoning in 14 men of the Royal Artillery in Quebec who drank tea made of three herbs (Ledum, Andromeda and Gaultheria). The symptoms were those of poisoning by oil of wintergreen contained in the gaultheria. In 1884, May reported 28 cases of salicylism out of 192 rheumatic patients who had taken either salicylic acid or sodium salicylate (Gross and Greenberg, 1948).

Hanzlik in 1913 summarised the clinical record of nearly 400 patients in whom toxic symptoms appeared after administration of various /

various salicylate compounds. Several thousands of cases of salicylate overdosage, mainly acetylsalicylic acid with or without fatalities have been reported in this century most of which have been reviewed by Gross and Greenberg (1948); Smith (1966); McCleave and Havill (1974); Mofenson and Greensher (1975); Bender (1975) and McQueen (1977).

(b) Terminology

The word "salicylate" is generally employed as a generic term for the class of compounds containing the salicyl radical C6H4 (OH). COO-, in application to the free acid, its salts, esters and ethers. The term "salicyl" is sometimes employed to designate the salicylic acid radical. Occasionally the word "aspirin" is used instead of acetylsalicylic acid (Smith, 1949). The term "salicylism" is employed for chronic intoxication of salicylate and the word "poisoning" for acute intoxication of salicylate.

(c) Chemistry

Of the three isomers of hydroxybenzoic acid, only the ortho compound (salicylic acid) came into medical use, because of its stronger action. Salicylic acid is so irritating that it can only be used externally, therefore, many of its derivatives have been synthesised for systemic use (Gross and Greenberg, 1948). Salicylic acid derivatives are divided into two large classes, namely "esters of salicylic acid" obtained by substitutions in the carboxyl group (e.g. methyl salicylate) and "salicylate esters of organic acids" in which the carboxyl group of salicylic acid is retained and substitution is made in the hydroxyl group (acetylsalicylic acid). /

acid). In addition, the effects of simple substitutions on the aromatic ring have been extensively studied, and new salicylate derivatives are still being synthesised (Flower et al., 1980). Diflunisal (2',4'-difluoro-4-hydroxy-3-biphenyl-carboxylic acid) is one of the new salicylic acid derivatives with a longer duration of action (Steelman, Cirillo and Tempero, 1978). Structural formulae of the salicylates are shown in Figure 1.

(d) Mode of action of salicylates

Although salicylates were known to inhibit a wide variety of reactions in vitro, no convincing relationship could be established with their known analgesic-antipyretic and anti-inflammatory effects (Flower et al., 1980). In 1971, Vane, and at the same time, Smith and Willis, reported that low concentrations of aspirin inhibited the enzymatic production of prostaglandins. The prostaglandins and related compounds are derived from 20-carbon essential fatty acids that contain three, four or five double-bounds. They fall into several main classes, designated by letters and distinguished by substitution on the cyclopentane ring. Those derived from arachidonic acid (the most abundant precursor in man) carry the subscript 2. Prostaglandins of E and Fα series are sometimes referred to as the "primary prostaglandins" which are the most common (especially E2 and F2α). Arachidonic acid is rapidly metabolised to oxygenated products by two distinct enzymatic mechanisms, a cyclooxygenase and a lypo-oxygenase. The unesterified precursor acids are oxygenated and cyclized by cyclooxygenase, to form the cyclic endoperoxide derivatives, prostaglandin G and prostaglandin H. These endoperoxides which are chemically unstable (half lives of 5 minutes at 37^{o}C and pH 7.5) are then isomerised into different products, prostaglandins E, /

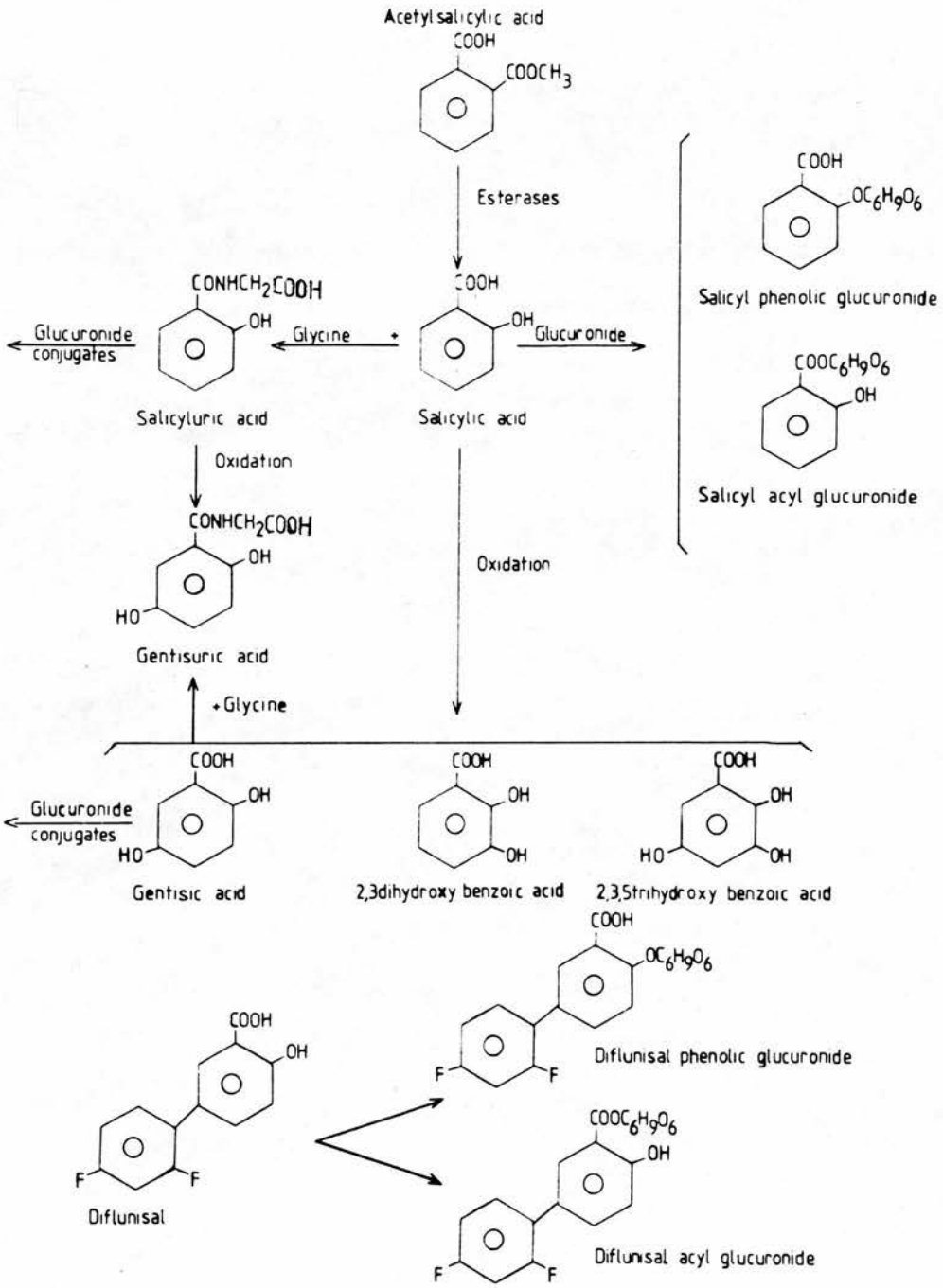

Fig 1 Metabolic pathways of acetylsalicylic acid and diflunisal (Modified from Milne 1962, and Steelman et al, 1978)

E, F or D. The endoperoxide prostaglandin H_2 is also metabolized into unstable and highly biologically active thromboxane A_2 and prostacyclin (prostaglandin I_2). Thromboxane A_2 has a very short half-life ($t_{\frac{1}{2}}$ = 30 seconds at 37°C and pH = 7.5) and breaks down into the stable thromboxane B_2 (Moncada, Flower and Vane, 1980).

All mammalian cell types studied (with the possible exception of erythrocytes) have microsomal enzymes for the synthesis of prostaglandins. Prostaglandins are always released when cells are damaged (Flower et al., 1980). Macrophages are a substantial source of prostaglandin E_2 formation and aspirin is the only non-steroidal anti-inflammatory drug which appears capable of impairing lymphocyte activation in therapeutic concentrations (Morley, 1977). Aspirin and related compounds prevent production of the prostaglandin endoperoxides by the inhibition of the cyclooxygenase enzyme and as a result, all the products below them in the metabolic pathway (Flower, 1974).

Following the isolation of cyclooxygenase, it was reported that aspirin quantitatively and selectively acetylated the oxygenase and interfered with its function. The in vitro inhibition by aspirin is about four times more potent than salicylic acid. The methyl ester of aspirin is also much less active, but gentisic acid is almost as effective as aspirin (Shen, 1979). Aspirin exerts two separate inhibitory effects on prostaglandin formation in vivo, a rapid action of the intact molecule on easily accessible tissue and a later action due to its metabolic conversion to salicylic acid. Salicylic acid inhibits prostaglandin biosynthesis in vivo as salicylate ion itself and there is no formation of subsequent active metabolites (Smith, Ford-Hutchinson, Walker and Slack, 1979).

The non-acetylating salicylate, diflunisal, exerts its anti-inflammatory /

anti-inflammatory and analgesic effects in man with a concomitant decrease of the excretion of 7∝-hydroxy-5,11, diketotetranoprostane-1-16, dioic acid, the major urinary metabolite of prostaglandin E1 and E2 (Steelman, Smith-Sibinga, Schulz, Vanden Heuvel and Tempero, 1976; Schulz, Perrier, Febert-Perret, Vanden Heuvel and Steelman, 1979)

(e) Pharmacological and toxicological effects of salicylates

1. Analgesia and antipyresis

The salicylates have lower maximal effects than do the opiate analgesics and hence are used only for pain of slight to moderate intensity. In a comparison of antipyretic and analgesic activity of aspirin with paracetamol, aspirin had some advantage over paracetamol for analgesia (Lovejoy, 1978). Whereas moderate doses of salicylate reduce an elevated body temperature, they also increase oxygen consumption and metabolic rate. In toxic doses, salicylates have a pyretic effect that results in sweating (Flower et al., 1980) and even malignant hyperthermia (Havill, 1974; Skjoto and Reikvam, 1979).

2. Respiration, acid-base and electrolyte disturbances

Salicylates stimulate respiration directly and indirectly. Full therapeutic doses of salicylates increase oxygen consumption and CO_2 production as a result of uncoupling of oxidative phosphorylation. The increased production of CO_2 stimulates respiration, but elevated alveolar ventilation balances the increased CO_2 production, therefore pCO_2 does not change or is reduced. If the salicylate is taken in overdosage it will gain access to the medulla and directly stimulate the respiratory centre. This results in marked hyperventilation with /

with increased respiratory minute volume (as much as ten-fold), low arterial pCO_2 and respiratory alkalosis (Ryder, Shaver and Ferris, 1945). Not only does salicylate increase pulmonary ventilation, it also results in enhanced sensitivity of the respiratory centre to arterial pCO_2 and concentration of hydrogen ions (Winters, White, Hughes and Ordway, 1959).

Although therapeutic doses of salicylate produce definite changes in the acid-base balance and electrolyte pattern (respiratory alkalosis), compensation promptly ensues by increased renal excretion of bicarbonate, sodium and potassium (compensated respiratory alkalosis). Ingestion of toxic doses of salicylate by children or occasionally adults is followed by changes in acid-base status. With very large doses, respiratory depression occurs and the enhanced production of CO_2 outstrips its alveolar excretion; consequently, arterial pCO_2 increases and pH decreases. Since the concentration of bicarbonate in plasma is already low due to its increased renal excretion, an uncompensated respiratory acidosis occurs superimposed however, on a true metabolic acidosis (Atkins, 1969). Respiratory alkalosis may not be observed in children. In 33 salicylate overdose patients, mostly infants and young children, significant reduction in the total CO_2 and plasma pCO_2 on admission were reported (Winters et al., 1959), whereas in 62 adults with severe salicylate poisoning, only 8 were found to be acidaemic, although the mean plasma salicylate concentrations of the acidaemic and non-acidaemic patients were similar (Proudfoot and Brown, 1969). In another study of 67 adults with salicylate intoxication, 25% had simple respiratory alkalosis (Gabow, Anderson, Potts and Schrier, 1978).

Pulmonary oedema /

Pulmonary oedema

The association of pulmonary oedema and salicylate intoxication in man was first reported by Granville-Grossman and Sergeant (1960) in a 32 year old man without heart disease who survived. Davis and Burch (1974) reported fatal non-cardiogenic pulmonary oedema in two adults and speculated that the cause might be salicylate-induced centrally mediated pulmonary oedema. Another fatal case of salicylate intoxication with pulmonary oedema was reported by Tweeddale (1974) and similar patients who survived by Tashima and Rose (1974), Hrnicek, Skelton and Miller (1974), Broderick, Reinke and Goldman (1976), Heffner, Starkey and Anthony (1979).

Respiratory distress syndrome

Adult respiratory distress syndrome in salicylate intoxication with extravasation of protein-rich fluid in the lungs, decreased pulmonary compliance, hypoxaemia and "white lungs" on chest X-ray was described by Sorensen (1979) in a 54 year old woman with a plasma salicylic acid concentration of 3.8 mmol/l (684 µg/ml). A similar case was reported by Thomas (1979) and a fatal respiratory distress syndrome in a two year old girl with a plasma salicylate concentration of 2.7 mmol/l (486 µg/ml) was described by Kahn and Blum (1979). Gullner (1979a) suggested that inhibition of prostacyclin biosynthesis by salicylate may be a contributory factor in the pathogenesis of respiratory distress syndrome and could explain the defect in haemostasis as well.

3. Renal effects

(1) /

(1) Acute effects

The effects of 7 to 200 mg/kg of acetylsalicylic acid given by intravenous infusion on renal function in the dog were investigated by Berg and Bergan (1976). They reported a significant dose-dependent decrease in sodium excretion (23%) and para-aminohippuric acid clearance (108.7 ml/min after 7 mg/kg of acetylsalicylic acid falling to 58.8 ml/min after 200 mg/kg) and also a 10% decrease in the renal tubular reabsorption of free water. The creatinine clearance was unchanged. Berg and Bergan offered the prostaglandin synthetase inhibitory effects of acetylsalicylic acid as one explanation of the effects on renal blood flow and sodium excretion. In fact the opposite effects of prostaglandins I_2. E_s and A_s on renal function in the dog have been reported (Moncada et al., 1980). Berg (1977a) also reported on the acute effects of acetylsalicylic acid on renal function in normal man. There was a significant decrease in the 24 hour sodium excretion and Na/K ratio, which was prominent during day time and when sodium intake was low. He also confirmed the antidiuretic effects of acetylsalicylic acid (which was most marked at night) and that the creatinine clearance was unchanged by aspirin treatment. Acute renal failure associated with therapeutic doses of aspirin and ibuprofen with patchy nonspecific ultrastructural changes in the tubular epithelium was reported by Kimberly, Sherman, Mouradian and Lockshin (1979). Further studies of the effect of acetylsalicylic acid on the creatinine clearance and inulin clearance in man revealed no significant changes (Nielsen, Rasmussen and Hilden, 1980; Muther and Benneth, 1980).

Renal "irritation" by salicylates was shown by an increase in the urine-cell-count (Scott, Denman and Dorling, 1963). The urine-cell- /

cell-count reached a maximum on the second and third day of aspirin treatment and later returned to normal even though the drug was continued. However, in a control study in which a new method was employed for the enumeration of renal tubular cells, 10 healthy volunteers (5 males and 5 females) took 3.6 g acetylsalicylic acid for 5 days. There was a dramatic rise in renal tubular cell output after a latent period of about 24 hours. This increase was not sustained, but by the fifth day the average renal-tubular-cell excretion was still more than four times the control value and in no volunteer had the count returned to normal (Prescott, 1965).

(2) Chronic effects

The obvious nephrotoxicity of salicylates and their probable primary role in the aetiology of analgesic nephropathy was emphasised by Prescott (1966a). A large number of patients with analgesic nephropathy have been described who took aspirin alone or with other analgesics without phenacetin (e.g. Prescott, 1966b; 1969; Clark and Linton, 1973; Wilson and Gault, 1977; Gokal and Matthews, 1977). Aspirin produces renal papillary necrosis more readily in animals than phenacetin (Molland, 1978; Nanra, Stuart-Taylor, De Leon and White, 1978). Molland (1978) suggested that the early papillary changes might be due to ischaemia, medullary blood flow being reduced as a result of aspirin's action as an inhibitor of prostaglandin synthesis.

The urinary excretion of N-acetyl-β glucosaminidase (NAG) has been used as a marker for identifying nephrotoxic drugs. A significant increase in the excretion of this enzyme was found in 83% of rheumatic patients receiving aspirin therapy. Single doses of 650, /

650, 1300 or 1950 mg of aspirin in healthy volunteers resulted in a significant increase in urinary NAG at 2 to 4 hours after the higher doses (Proctor and Kunin, 1978). In a comparative nephrotoxicity study of aspirin and diflunisal in osteoarthritic patients, NAG excretion increased in a dose-related manner with a relatively greater increase in the aspirin group (Dieppe, 1978).

Acute interstitial nephritis in a patient with aspirin hypersensitivity was reported by McLeish, Senitzer and Gohara (1979). The nephrotoxicity and hepatotoxicity of antipyretic analgesics (including aspirin) was reviewed by Prescott (1979a).

4. Hepatic effects

Prolonged administration of salicylates, particularly in rheumatic patients, may cause liver failure and encephalopathy which simulate Reye's syndrome (Lyon and Nevins, 1974; Daum, Zunker and Cohen, 1976). In animal experiments salicylates produced elevation of transaminases and fatty changes, but the changes differed from those seen in Reye's syndrome (Linnemann, Ueda, Hug, Schaeffer, Clark and Schiff, 1979). However, there is no doubt that chronic salicylate administration, even with plasma salicylate concentrations less than 25 mg/100 ml, may induce hepatotoxicity (Bernstein, Singsen, King and Hanson, 1977; Schaller, 1978; Kanada, Kolling and Hinddin, 1978). A case of aspirin hepatotoxicity and disseminated intravascular coagulation was also reported (Sbarbaro and Bennett, 1977).

5. Gastrointestinal effects

The ingestion of salicylate may cause epigastric distress, nausea, /

nausea, vomiting, errosive gastritis and gastrointestinal haemorrhage. The relationship between aspirin ingestion and massive gastrointestinal haemorrhage was reviewed by Shirley (1977) who was unconvinced of a cause and effect relationship. There has even been a suggestion that aspirin consumption may not necessarily be the cause of bleeding but an associated phenomenon (Langman, 1977). However, in an investigation of gastrointestinal blood loss (Vakil, Kulkarni, Kulkarni, Mehta, Gharpure and Pispati, 1977) and an endoscopic study of gastrointestinal injury (Vakil, Shah, Dala, Wagholikar and Pispati, 1977) in 24 healthy volunteers treated with non-steroidal anti-inflammatory agents it was demonstrated that aspirin produced the greatest degree of blood loss and most severe mucosal damage. This was also confirmed by histopathological findings. Similar studies were reported by Silvosa, Ivey, Butt, Lokard, Holt, Sisk, Baskin, Mackercher and Hewett (1977), Mielants, Veys, Verbruggen and Schelstraete (1979), Cohen (1979), and Faivre, Faivre, Lery, Ducluzeau, Moulinier and Paliard (1979). However, intravenous aspirin did not produce detectable histological mucosal damage in healthy subjects and it was concluded that high blood salicylate concentrations do not acutely damage gastric mucosa (Ivey, Paone and Krause, 1980). Hunt and Fisher (1980) reported that despite a rise in plasma salicylate concentrations from 55 to 74 mg/l the rate of blood loss into the gastric lumen declined.

In a comparative study of diflunisal versus aspirin on their effects on faecal blood loss in the presence and absence of alcohol, diflunisal did not significantly increase blood loss, while aspirin had a significant effect, which was enhanced by the addition of alcohol (De Schepper, Tjandramaga, Verhaest, Daurio and Steelman, 1978).

6. /

6. Effects on the blood

Aspirin has multiple adverse effects on haemostasis (Prescott, 1977). It may cause anaemia through different mechanisms. The commonest is simple iron deficiency anaemia caused by occult blood loss from the gastrointestinal tract, but haemolytic, macrocytic and aplastic anaemias have also been documented (Prescott, 1975; Eldar, Aderka, Shoenfeld, Lirni and Pinkhas, 1979). Hypoprothrombinaemia may occur if large doses of salicylates are taken for a long period, but it is rarely responsible for serious bleeding and responds to injection of vitamin K1 (Prescott, 1968).

There are many reports of the effects of acetylsalicylic acid on platelet function (e.g. Evans, Packam, Nishizawa, Mustard and Murphy, 1968; Weiss, Aledort and Kochwa, 1968; O'Brien, 1968; Morse, 1977; Rajah, Penny, Crow, Pepper and Watson, 1979). Acetylsalicylic acid interferes primarily with the second phase of platelet aggregation. The effects of aspirin on platelet aggregation as a function of dosage and time were reported by Paccioretti and Elock (1980). They concluded that platelet aggregation inhibition by a single oral dose of 81 mg aspirin or more may be expected to persist for the lifetime of the platelets affected. The differential inhibition of prostacyclin production and platelet aggregation by aspirin was studied by Masotti, Galanti, Poggesi, Abbate and Neri Serneri (1979) and Pareti, D'Angelo, Mannucci and Smith, 1980; O'Brien (1980). Masotti et al. pointed out that inhibition of platelet cyclo-oxygenase occurs with smaller dose of aspirin (3.5 mg/kg) and lasts longer than inhibition of vessel-wall cyclo-oxygenase. Hoogendijk and Ten Cate (1980) reported different results in patients and concluded that results obtained in healthy volunteers may not be applicable to patients. /

patients. Huijgens, Imandt and Van Den Berg (1980) also concluded that 3 mg/kg of aspirin seems to give maximal platelet aggregation inhibition. Following ingestion of 650 mg of aspirin, cyclo-oxygenase activity was inhibited 95% within 45 minutes. Enzyme activity was observed to increase within 8 hours and reached 10% of control level by 24 hours. It has been suggested that only circulating platelets are affected by acetylsalicylic acid (Ali, McDonald, Thiessen and Coats, 1980).

Although the bleeding time was prolonged significantly by aspirin relative to control values (Stuart, Miller, Davey and Wolk, 1979) it was still within the normal bleeding time range in the general population.

A single oral dose of 100 mg aspirin reduced serum thromboxane B_2 by 98% during the first hour and single doses of 100-400 mg resulted in 94-98% inhibition after 24 and 48 hours which returned to control levels within a time course consistent with platelet turnover (Patrono, Cialiattoni, Pinca, Pugliese, Castrucci, De Salvo, Satta and Peskar, 1980).

7. Central Nervous System Effects

The effects of salicylates on the central nervous system are not only prominent in overdosage, but are also reported as common side effects with therapeutic doses. Among 2,391 hospitalised patients receiving plain aspirin tablets, 1.2% had central nervous system side effects (second only to heartburn and nausea). Of these tinnitus was reported most often (0.8%) and deafness occurred in 0.3% of cases and was associated with high doses (Miller and Jick, 1977). In another study, aspirin-induced deafness was found to be dose-related (Miller, 1978).

8. Other effects

Large doses of salicylates have been reported to increase cardiac output, and the plasma volume may be increased as a result of salt and water retention (Prescott, 1972). Allergic reactions (e.g. asthma, urticaria, shock, collapse and angioneurotic oedema), teratogenicity, endocrinal and many other side effects are also documented (e.g. Seltzer, 1973; Prescott, 1978, 1979b and 1980; Hunter, Dorward, Knill-Jones and Gunn, 1978).

Salicylate poisoning

Acute salicylate intoxication usually occurs following intentional ingestion of aspirin in overdosage in adults and accidental or therapeutic poisoning in children (Pierce, 1974; Buchanan, 1975). Methylsalicylate (oil of wintergreen) is more toxic than aspirin. As little as 5 ml of oil of wintergreen will kill a child and can produce a plasma concentration over 700 µg/ml in an infant, which is equal in toxic effect to 15 adult aspirin tablets (Locket, 1973). Intoxication following percutaneous absorption of salicylic acid has also been reported (Smith and Lyons, 1980; Davies, Briffa and Greaves, 1979). Salicylate poisoning occurred in a 21-month-old boy following the use of a teething ointment containing 8.7% choline salicylate (Paynter and Alexander, 1979). Chronic salicylate poisoning in children during therapy with aspirin is aggravated by the dose-dependent kinetics of the drug (Done, 1978). Two cases of therapeutic salicylate poisoning in children were reported by Mitchell (1979). Self-medication for treating "knee jerk" in a 52 year old woman caused severe salicylate intoxication (Edward and /

and Taylor, 1980). Children are much more susceptible to the toxicity of salicylate than adults (Done, 1978). Many children have died following therapeutic doses or accidental overdosage of aspirin (Krasnoff and Bernstein, 1947; Greenberg, 1950; Shannon, 1965; Craig, Ferguson and Syme, 1966; Whitehall, 1973; Done, 1978). Between 10% and 20% of adult self-poisoning in Edinburgh involves aspirin (Proudfoot and Park, 1978).

Clinical manifestations in 49 children with salicylate poisoning included hyperpyrexia, respiratory alkalosis, dehydration, metabolic acidosis and hypernatraemia. The fatal cases were children under two years of age (Segar and Holliday, 1958). Acid-base changes in salicylate poisoning are complex and depend on the severity and duration of the intoxication, individual sensitivity and age. Metabolic acidosis is very common in children and may dominate the respiratory alkalosis which is usually seen in severe salicylate poisoning in adults (Winters et al., 1959; Temple, 1978). Experiments in mice and rats using radioactive and non-radioactive techniques showed that brain salicylate concentrations were almost doubled in acidaemic compared with the alkalaemic animals (Hill, 1973). However, there are reports of disturbed cerebral function with high plasma salicylate concentrations in poisoned adults who were not acidaemic (Levy, 1968; James and Matrinak, 1975; Proudfoot and Prescott, 1977).

Clinical features of salicylate poisoning include tinnitus, deafness, sweating, vasodilation and hyperventilation. Contrary to the belief of many doctors, loss of consciousness is very rare in salicylate poisoning, and when it does occur, intoxication is severe and likely to be fatal (Bender, 1975; Proudfoot and Prescott, 1977; McQueen, /

McQueen, 1977; Rumack, 1979). Although salicylates cause hypoprothrombinaemia and gastric erosions, severe bleeding is a rare complication of the acute poisoning (Proudfoot and Prescott, 1977). Hypoglycaemia and convulsions seem to occur almost exclusively in young children (Prescott, 1975; Bray and Gardiner, 1977).

The diagnosis of salicylate poisoning is usually easy, but can easily be overlooked. The diagnosis of salicylate intoxication was suspected in a 37-year-old man because of the results of serum electrolyte and arterial blood gas analysis (Greenbaun, Togba, Becker and Grace, 1974). In a study of 73 consecutive adults hospitalised with salicylate intoxication, 27% of the patients were undiagnosed as long as 72 hours after admission, although the initial clinical findings and laboratory data in patients not diagnosed on admission did not markedly differ from the findings in patients diagnosed on admission. However, the patients with delayed diagnosis were older, rarely had a previous history of drug overdose and more often became accidentally intoxicated through therapeutic use of aspirin (Anderson, Potts, Gabow, Rumack and Schrier, 1976). Unusual abdominal complications (blind-loop syndrome) were reported in a suicidal 67-year-old woman following ingestion of aspirin, amitriptyline and diazepam (Mehta, Mehta and Matthew, 1978). Springer and Groll (1980) reported a nearly fatal salicylate overdose occurring suddenly after gastric retention of enteric-coated aspirin, ingested over a 14-week period.

Measurement of the plasma salicylate concentrations on admission and its subsequent rate of decline provides a valuable indication of the severity of poisoning and a useful guide to treatment (Brown, Cameron and Matthew, 1967). There appears to be a direct relationship between dose absorbed and plasma concentrations two hours after /

after ingestion (McCleave and Havill, 1974), but salicylate concentrations may not always correlate well with the clinical severity of intoxication (Done, 1960; Prescott, Roscoe and Forrest, 1973). However, Done (1960) found a better correlation between a theoretical zero-time concentration and clinical severity of salicylate intoxication in children. In 59 subjects experiencing tinnitus the serum salicylate level was invariably greater than 196 μg/ml (Mongan, Kelly, Nies, Porter and Paulus, 1973).

Treatment of salicylate poisoning

Therapy of salicylate poisoning should be aimed principally at replacement of fluid and electrolytes, correction of acidaemia, prevention of further salicylate absorption and enhancement of salicylate elimination (Temple, 1978). As much as 20 g of salicylate was recovered by gastric aspiration and lavage in one case, nine hours after ingestion of 200 aspirin tablets (Matthew, Mackintosh, Tompsett and Cameron, 1966). In another report, Matthew (1970) recommended gastric aspiration and lavage for acute poisoning using a simple rule of a 4-hour interval since ingestion with the exception of salicylate in which the interval is extended to 12 hours. Gastric lavage with sodium bicarbonate solution has been used for removing enteric-coated aspirin (Sogge, Griffith, Sinar and Mayes, 1977). Despite gastric lavage, the plasma salicylate concentrations may continue to rise for several hours. Facial petechiae may be noted, particularly on the eyelids, probably as a result of the effects of aspirin on platelet and capillary function and the added mechanical effects of the lavage (Proudfoot and Prescott, 1977). Emetics, /

Emetics, such as ipecac, (but not sodium chloride) may be used instead of gastric lavage and should be administered as soon as possible. A 3 year-old-boy was given salt as an emetic and later gastric lavage with a saline solution following accidental ingestion of aspirin. He became comatose and died with severe persistent hypernatraemia and hyperchloraemia although his plasma salicylate concentrations declined from 509μg/ml to 90μg/ml (Barer, Hill, Hill and Martinez, 1973). Activated charcoal may also be given (Levy and Tsuchiya, 1969). Although it will reduce aspirin absorption if given at the same time it is largely ineffective if given more than 1 hour after ingestion (Levy and Tsuchiya, 1972). Charcoal-sorbitol mixture and kaolin pectin are both less effective in reducing aspirin absorption than charcoal slurry (Mayersohn, Perrier and Picchioni, 1977; Juhl, 1979).

Different methods of treatment have been used to enhance salicylate elimination. The effects of forced diuresis, alkaline diuresis, forced alkaline diuresis and diuretics on salicylate kinetics are reviewed in detail later. Osmotic diuresis by mannitol was used in three patients with severe salicylate poisoning giving a plasma elimination half-life of 12 hours (Ghose and Joekes, 1964). Mannitol was found to have a transient diuretic effect on the acute symptoms associated with fluid retention in salicylate poisoning, but only strict fluid restriction resulted in a prompt and satisfactory diuresis (Temple, George, Done and Thompson, 1976). The use of acetazolamide for salicylate poisoning, in the absence of definitive clinical and chemical data, cannot be recommended (Winters, 1959). Acetazolamide and intravenous fluids were administered to 27 patients with salicylate poisoning (Feurestein, Finberg /

Finberg and Fleishman, 1960). However, Bonginovanni (1960) criticised the use of acetazolamide in this study in patients with plasma salicylate concentrations less than 350 μg/ml (maximum therapeutic concentration), which accounted for its apparent effectiveness. Finberg (1960) replied that the time interval from ingestion of the drug is more relevant. Although acetazolamide enhances salicylate elimination, it might aggravate the profound acidosis present in most children with severe salicylate poisoning (Reimold, Worthen and Reilly, 1973; Jewett, 1973).

Haemodialysis for aspirin poisoning was first reported by Doolan, Walsh and Wishinsky (1951) and they recommended early treatment. Dukes, Blainey, Cumming and Windowson (1963) compared simultaneous haemodialysis and forced diuresis with forced alkaline diuresis for the treatment of salicylate poisoning and both were effective. The effects of charcoal haemoperfusion and haemodialysis for severe salicylate poisoning in laboratory animals have been compared (Hill, 1973).

f - Salicylate pharmacokinetics and the use of forced alkaline diuresis for poisoning

1. Absorption

Salicylates are almost always taken orally (Levy, 1962) and the most common form is acetylsalicylic acid, a weak organic acid with a pKa value of 3.5. Its gastrointestinal absorption occurs predominately by passive diffusion of non-ionised molecules across the biological membrane (Milne, Scribner and Crawford, 1958; Levy and Leonard, 1966). In the acidic gastric contents (pH 1-2) acetylsalicylic acid is nonionised, thus it enters the mucosal cells where /

where it becomes ionised (pH 7.0). Ionised acetylsalicylic acid passes slowly into neighbouring compartments such as endothelial cells and capillaries (Brune, Graf and Rainsford, 1977). Three factors (gastric emptying, aspirin dissolution and intragastric pH) which influence acetylsalicylic absorption were studied in human subjects and dogs. The major portion of the salicylate in the blood during the first 20 minutes came from the stomach. The impedance of gastric emptying did not affect, but low pH and rapid dissolution accelerated acetylsalicylic acid absorption (Truitt and Morgan, 1964). Pottage, Nimmo and Prescott (1974) showed that the pH-partition theory did not hold in clinical practice. They found that achlorhydric patients absorbed the drug more rapidly than healthy volunteers.

Levy (1961) showed that the in vivo absorption rates of different commercial aspirin tablets were proportional to the in vitro dissolution rate. Leonard (1963) also studied the influence of solubility on the rate of gastrointestinal absorption of aspirin in a total of 168 experiments in 55 normal subjects. He demonstrated that plain aspirin was absorbed relatively slowly and maximum blood concentrations of salicylate were not attained by 60 minutes. Buffered aspirin preparations were more rapidly absorbed with peak plasma concentrations at 30 to 45 minutes and for effervescent tablets the peak was at 20 to 30 minutes. Similar results were obtained with aspirin dissolved in hot water. In another study, it was found that buffered aspirin in a test meal (pH 7.0) emptied more rapidly from the stomach than a test meal containing unbuffered (pH 2.8) aspirin. After 10 minutes at least ten times less was absorbed from the buffered solution (Cooke /

(Cooke and Hunt, 1970) demonstrating rapid gastric absorption of unbuffered aspirin. Chiou and Onyemelukwe (1974) showed that there were no statistically significant differences in cumulative salicylate urinary excretion at 3, 6, 12 and 24 hours, between five different brands of buffered aspirin tablets. Food tends to reduce the rate of absorption of acetylsalicylic acid (Koch, Schultz, Wills, Hallquist and Welling, 1978). The absorption kinetics following oral administration of an aqueous solution of 650 mg aspirin were studied by Rowland, Riegelman, Harris and Sholkoff (1972). They concluded that absorption was a first-order process, with a half-life ranging from 4.5 to 16 minutes suggesting that absorption is predominately from the stomach. Only 68% of the dose reached the peripheral circulation intact and the remainder was hydrolysed during absorption by esterases in the gut wall.

Drug interactions may enhance absorption (Prescott, 1973). Plasma salicylate concentrations were greater following oral administration of the codeine-containing preparation, Safapryn-Co than from the identical preparation without codeine, Safapryn (Nimmo, King and Prescott, 1979; Bradbrook, Morrison, Rodgers and Spector, 1979).

In summary, unbuffered aspirin appears to be absorbed more rapidly from the stomach. However, gastric emptying is more rapid with buffered aspirin, which is then absorbed from the small intestine.

2 - Distribution and Metabolism

Over the dose range 0.3 to 1.2 g of acetylsalicylic acid, the area under the acetylsalicylic acid concentration-time curve was proportional to the dose administered. Salicylic acid was shown to be /

be the exclusive metabolite of acetylsalicylic acid. Following absorption, the liver and perhaps the kidney were proposed as the principal sites of hydrolysis of acetylsalicylic acid. After intravenous administration of 650 mg acetylsalicylic acid or 500 mg salicylic acid, the first exponent plasma elimination half-life was 2 - 5 minutes for both compounds, whereas the second exponent was 13 - 19 minutes and 3.5 - 4.5 hours for acetylsalicylic acid and salicylic acid respectively (Rowland and Riegelman, 1968; Thompkins and Lee, 1969). The plasma elimination half-life of salicylate is dose-dependent. An average half-life of 20 hours (15 - 29 h) in 17 intoxicated children with salicylate was reported by Done (1960).

Levy and Leonards (1966) pointed out that relatively slow diffusion across certain tissue barriers may result in lag effects, with maximum salicylate concentrations occurring at different times in different tissues. High concentrations of acetylsalicylic and salicylic acids are found in the glandular region of the stomach and the kidney tubules of the rat as well as in inflamed tissue (Brune, 1977). Even at high plasma salicylate concentrations very little is present in the human erythrocytes (Smith, Gleason, Stoll and O'Gorzalek, 1946).

The major metabolite of salicylic acid is the glycine conjugate, salicyluric acid. Salicylic acid forms an ether or phenolic glucuronide and an ester or acyl glucuronide. In addition, a small fraction is oxidised to gentisic or 2,5 dihydroxybenzoic acid and an even smaller fraction to 2,3 dihydroxybenzoic and 2,3,5 trihydroxybenzoic acids (Milne, 1962) (Fig.1). The formation of salicyluric acid from salicylic acid reaches a maximal rate when the total amount of salicylate in /

in the body exceeds 300 mg (Levy, 1965 a). Administration of glycine has no effect on the formation of salicyluric acid, but benzoic acid has a pronounced inhibitory effect and surprisingly this is not prevented by the co-administration of glycine (Amsel and Levy, 1969). Inter-individual differences in glycine conjugation in 58 male and 27 female healthy volunteers were largely normally distributed, but with a 12-fold range (Caldwell, O'Gorman and Smith, 1980). Phenolic glucuronide formation is also capacity-limited (Hollister and Levy, 1965; Levy, Tscuchiga and Amsel, 1972).

The apparent volume of distribution of salicylate is dose-related. In children it ranged from 162 to 345 ml/kg and was larger at the higher doses (Levy and Yaffe, 1974). Presumably the larger distribution at higher doses is due to the highly concentration-dependent nature of salicylate protein binding, vasodilation, higher cardiac output and increasing uptake into tissues as a result of acidosis.

The kinetics of salicylate metabolism were the same in patients with rheumatoid arthritis and non-inflammatory backache. (Gibson, Zaphiropoulos, Grove, Widdop and Berry, 1975). Neither the plasma salicylate concentration-time curves nor the plasma elimination half-lives differed in young and elderly subjects in one report (Melander, Bodin, Danielson, Gustafsson, Hugland and Westerland, 1978), but in another study the plasma elimination half-lives and the volume of distribution of salicylate were significantly lower in the young subjects (Cuny, Royer, Mur, Serot, Faure, Netter, Millard and Penin, 1979).

Time-dependent changes in the pharmacokinetics of acetylsalicylic acid were studied following a single 1500 mg oral dose.

The /

The bioavailability of the drug was significantly greater when it was administered at 06.00 than at 18.00 or 22.00 hours (Markiewicz and Semenowicz, 1979).

The effects of chronic administration of aspirin (3.9 g/day) were studied in eight subjects. Plasma and salivary salicylate concentrations declined significantly after peak levels were achieved between days 3 and 10, possibly due to an induction of salicylurate formation. The mean salicylurate excretion increased with time during the study, although the change was not statistically significant (Rumble, Brooks and Roberts, 1980).

The plasma protein binding of salicylate will be reviewed in Chapter 3, Section II.

3 - Excretion

The renal excretion of unconjugated salicylate involves glomerular filtration, active tubular secretion and tubular reabsorption (Schachter and Manis, 1958).

The rate of elimination of salicylate following oral single and multiple doses of acetylsalicylic acid in healthy male volunteers was studied (Bedford, Cummings and Martin, 1965) and the excretion patterns were as follows :

(i) an initial period when drug absorption, distribution and metabolism affect the rate of excretion

(ii) an intermediate period when the rate of excretion is by simultaneous first-order and zero-order processes

(iii) a final period when all metabolites are formed with apparent first-order kinetics

Practically all of a dose of acetylsalicylic acid can be recovered /

recovered in the urine as salicylic acid and its various metabolites and elimination is slower at high than at low doses (Levy, 1965b; Levy and Yaffe, 1968). The rate of excretion of acetylsalicylic acid metabolites depends on factors such as urine pH and flow rate (Davison, 1971).

Urinary excretion kinetics of salicyluric acid following oral administration of 640 mg acetylsalicylic acid in healthy volunteers and intravenous administration of salicyluric acid in man (196 mg) and dog (9 mg/kg) were studied. The mean urinary excretion rate constant was found to be 0.109 in man and 0.0139 in the dog (Elliott, 1966). In another study, the kinetics of salicyluric elimination in man after intravenous (97.5 mg) and oral (150 mg) salicyluric acid and salicylic acid (1 g orally 3 hr earlier) followed by benzoic acid (3.2 g orally) as the blocker in healthy volunteers resulted in a rate-limiting formation step in the excretion of salicyluric acid following salicylic acid administration (Levy, Amsel and Elliott, 1969).

The urinary excretion of gentisic acid in man accounted for 0.6% of a 0.32 g and 1.1% of a 1.28 g dose of oral acetylsalicylic acid, suggesting that the excretion of gentisic acid is dose-dependent (Boreham and Martin, 1969).

4 - Effects of urine pH and flow rate on salicylate excretion following therapeutic doses

According to Hanzlik (1926), Fleisher in the last century reported that the administration of sodium bicarbonate shortened the "period of elimination" of salicylate from 36 to 14 hours and Ehrmann made a similar claim, but this was not confirmed by Hanzlik, Scott and Thoburn. However, the administration of 3 g sodium bicarbonate every 3 hours together with /

with 2 - 3 g sodium salicylate every 6 hours in rheumatic patients lowered plasma salicylate levels. The renal excretion and clearance of free salicylate increased as the urine pH rose above 7.0 (Smith, Gleason, Stoll and Ogorzalek, 1946). In another study, oral administration of 7.8 g sodium bicarbonate within 3.5 hours of a single oral dose of acetylsalicylic acid in healthy volunteers increased the mean urinary excretion of salicylate from 925 to 1501 mg (Lester, Lolli and Greenberg, 1946). Hoffman and Nobe (1950) also studied the influence of urinary pH on the renal excretion of salicyl derivatives during aspirin therapy and concluded that when sodium bicarbonate was administered in quantities sufficient to alkalinise the urine, the free salicylate clearance was increased by a factor of 3-13.

The effect of changes in urine flow rate and pH on salicylate excretion was studied in two healthy adult male volunteers and three patients (one with normal renal function and two with chronic glomerulonephritis and uraemia). There was a good correlation between the urine pH and free salicylate clearance, less effect with water diuresis and a moderate effect with osmotic diuresis. Salicylate excretion was lower in the patients with moderate uraemia (McPherson, Milne and Evans, 1955).

The effects of infusion of 5 g sodium bicarbonate in 600 ml (3 g in 100 ml in 10 minutes and 2 g in 500 ml as sustaining infusion) on the simultaneously determined renal clearances of salicylate, inulin and p-amino-hippurate were studied in 10 normal human subjects by Gutman and Sirota (1955) with the following results :

1. /

1. Inulin and p-aminohippurate clearances were not consistently affected by infusion of sodium salicylate alone or in combination with sodium bicarbonate.
2. The free salicylate/inulin clearance ratios ranged from 5% to 60% and as the urinary pH rose, the salicylate clearance increased approximately three-fold while the net tubular reabsorption of free salicylate decreased correspondingly. When the urine pH exceeded 7.5 more salicylate was usually excreted than filtered, implying active tubular secretion. It was suggested that the renal elimination of salicylate is a complex process involving glomerular filtration, tubular conjugation, tubular secretion and tubular reabsorption.

The administration of 4 g of sodium bicarbonate in 13 subjects taking 4 g of aspirin daily resulted in a statistically significant decrease in plasma salicylate concentrations (Levy and Leonard, 1971).

Antacids may increase urine pH and can lower steady-state plasma salicylate concentrations under clinical conditions (Levy, 1978).

5 - Effects of forced alkaline diuresis in aspirin poisoning

Intravenous administration of 3.5 to 5 mEq/kg of sodium bicarbonate resulted in an alkaline urine in 15 of 18 children with salicylate poisoning. There was a significantly more rapid fall in serum salicylate concentrations than in control patients who received fluid alone (Oliver and Dayer, 1960). The possible therapeutic usefulness of forced diuresis for the treatment of salicylate poisoning was reported by Cumming (1961) and confirmed by Clemmesen, Myschetzky and Lassen (1962).

A /

A forced alkaline diuresis regime consisting of infusion of 500 ml of 0.9% sodium chloride, 5% dextrose and 2% sodium bicarbonate in rotation at 2 litres per hour for 8 hours gave results apparently comparable to haemodialysis in producing clinical and biochemical improvement in severe salicylate poisoning (Dukes et al., 1963). Intravenous sodium chloride and sodium bicarbonate solution produced an average urine-flow rate of 6.9 ml/min at a mean pH of 7.5 in patients with aspirin poisoning and resulted in a diminution of serum salicylate concentration to one-half of the original values in a mean time of 7.5 hours. Serum potassium concentrations also fell during treatment from a mean of 5.1 to 3.2 mEq/l (Cumming, Dukes and Widdowson, 1964). In 28 patients suffering from moderate to severe salicylate poisoning, forced alkaline diuresis produced few complications and there was no mortality (Mackintosh and Matthew, 1964). It was suggested that with the use of 5% sodium bicarbonate in 3% glucose additional chloride (as potassium chloride) might be given to offset losses due to sweating and vomiting (Garber, 1964). Beveridge, Forshall, Munro, Owen and Weston (1964) infused one litre of M/6 sodium bicarbonate and one litre of 5% dextrose during the first 2 hours, followed by smaller amounts to maintain an alkaline urine with a urine flow rate of over 500 ml/hour. This regime was not effective in some patients and was criticised by Lawson, Mackintosh and Matthew (1964) for the use of such a modest infusion.

Lawson, Proudfoot, Brown, Macdonald, Fraser, Cameron and Matthew (1969) compared different regimes of forced diuresis for aspirin poisoning in adults, with clinical and biochemical monitoring. They concluded that although forced alkaline diuresis (0.5 litre of 0.9% /

0.9% saline, 5% laevulose and 1.26% sodium bicarbonate in rotation at a rate of 2 litres per hour, with one gram of potassium chloride added to each 0.5 litre bottle from the fifth onwards) produced the quickest fall in plasma salicylate concentrations, its use was complicated by hypokalaemia despite the considerable potassium supplements and there was also a potentially dangerous rise in arterial pH. They recommended forced "cocktail" diuresis (a mixture of 0.5 litre of normal saline, one litre of 5% laevulose, 0.5 litre of 1.26% sodium bicarbonate and 3 gram of potassium chloride, given a 2 litres per hour for 3 hours) as the treatment of choice for acute salicylate poisoning in adults, and it could be safely undertaken without biochemical control. Infusion of the "cocktail" failed to alkalinise urine (pH > 7.0) which is possibly due to oxidation of laevulose in the alkaline solution.(Conn and Stumpf, 1972). Hypokalaemia induced by the forced alkaline diuresis may be due to the late infusion of potassium (from the fifth bottle onwards).

A 36 year old man with severe salicylate poisoning (blood salicylate concentration over 1300 µg/ml) was treated successfully with a different regime of forced alkaline diuresis (immediate intravenous administration of 150 mEq of sodium bicarbonate followed by the rapid infusion in rotation of sodium bicarbonate, normal saline and 5% dextrose). During the first 12 hours, 14 litres of fluid were administered and the urine output was 10.3 litres ; 190 g of mannitol was given in attempts to counter fluid retention (Savage, Ward, Simpson and Cohen, 1969). They did not consider the great insensible loss due to hyperventilation and sweating in severe salicylate poisoning and if the patient had been weighed before infusion and 24 hr. later, it could have been realised that there was no indication for the mannitol.

The effects of alkaline mannitol diuresis (10 litres containing 348 mEq of sodium bicarbonate and 475 g of mannitol) and forced alkaline diuresis (10 litres containing 360 mEq of sodium bicarbonate and 365.5 g of dextrose) were compared in two groups of patients with moderate to severe salicylate poisoning. Mannitol did not increase the excretion of salicylate, although there was a smaller fall in the serum potassium and rapid removal of salicylate for a smaller rise in serum pH than with the forced alkaline diuresis alone (Prowse, Pain, Marston and Cumming, 1970).

Morgan, Bennett and Polak (1968) proposed the administration of one litre of 10% mannitol in the first hour and one litre of M/6 lactate over the next two hours, and subsequently compared it with an acetazolamide-bicarbonate regime in which 250 mg i.v. acetazolamide followed by one litre of 1.4% sodium bicarbonate was given in the first hour and one litre of 1.4% sodium bicarbonate plus 20 mEq potassium chloride in the second hour (Morgan and Polak, 1969). The two groups of patients had serum salicylate concentrations ranging from 440 to 880 µg/ml and the relationship between urine pH and salicylate clearance was the same in both groups although they were higher with the acetazolamide-bicarbonate treatment. The influence of urine flow rate on the relationship between salicylate clearance and urine pH was also similar (Morgan and Polak, 1971). Since there was a highly significant negative relationship between salicylate elimination half-life and the renal clearance and it is known that acetazolamide may depress tubular secretion of salicylate by competitive inhibition (Weiner, Washington and Mudge, 1959) the superior effects of the acetazolamide-bicarbonate treatment should be mainly due to the bicarbonate only.

Water /

Water diuresis (0.225% saline solution with 2.5% glucose and 20 mEq/litre potassium chloride, given at a rate of 2 ml/minute), forced alkaline diuresis (the same regime as the water diuresis, but including sodium bicarbonate to increase the urine pH above 7.5) and forced alkaline diuresis plus acetazolamide (10 mg/kg) were compared in three groups of young dogs with salicylate intoxication (blood salicylate concentration 1000 µg/ml). Blood salicylate concentrations fell at about the same rate in all three groups. Urinary salicylate excretion and pH increased rapidly following acetazolamide while there was a three-hour delay in salicylate excretion after bicarbonate and it did not increase at all with water diuresis. Although the rapid action of acetazolamide favours its use in the treatment of salicylate poisoning, the profound acidosis present in most children precludes its administration (Reimold et al., 1973).

Prescott (1974) stressed the haemodilution and changes in the volume of distribution of salicylate which occur during forced alkaline diuresis and believed that the amounts of drug claimed to have been removed by this method of treatment may be overestimated.

6 - Effects of forced alkaline diuresis with loop diuretics in aspirin poisoning

Berg (1977b) compared the effects of frusemide and bumetanide (20 mg and 0.5 mg 2 hourly) in two groups of patients (9 and 8 respectively) with plasma salicylate concentrations of 300-600 µg/ml who were receiving forced alkaline diuresis (0.9% saline, 5% dextrose and 1.4% sodium bicarbonate with potassium and calcium chloride supplements infused at a rate of 250-300 ml/h for 16-48 hours). The two groups /

groups had equivalent 24 hour urine outputs (about 6 l/24 hours), but the patients on frusemide retained significantly less fluid than the other group (means of 696 and 1152 ml respectively). The urine flow rate was at least as much as could be achieved by osmotic diuretics and since the latter have more side effects, they should not be used for forced diuresis. Dehydration was a common problem in patients who had ingested aspirin more than 12 hours before admission and Berg concluded that diuresis may prevent rehydration. However, in the average adult patient who is admitted early after aspirin ingestion, sodium and water retention is more likely and forced alkaline diuresis with loop diuretic should be used with careful supervision of fluid balance.

Infusion of 75 ml of 20% mannitol and 40 mg frusemide followed by 500 ml of 5% dextrose plus 50 mEq sodium bicarbonate, 500 ml of 5% dextrose plus 25 mEq potassium chloride and 500 ml of normal saline in rotation at a rate of 30 ml/kg/hr. was recommended (McQueen, 1977) as the urgent treatment for salicylate poisoning, but there is no evidence to support the efficacy of this regime.

Interaction between salicylate and two loop diuretics (frusemide and piretanide) was studied in four healthy subjects (3 M and 1 F) by Valette and Apoil (1979). When lysine acetylsalicylic acid was given before frusemide (but not piretanide), there was a significant decrease in the diuresis and sodium excretion and an increase in potassium excretion. They suggested different mechanisms of interaction such as prostaglandin synthetase inhibition and pharmacokinetics related to the renal uptake and transport of the diuretics.

In /

In unpaired and paired studies in man, the effects of 1000 and 1500 mg of intravenous acetylsalicylic acid on the glomerular filtration rate, urinary sodium and potassium excretion and osmolality were studied with and without 40 and 10 mg of intravenous frusemide (Bartoli, Arras, Faedda, Soggia, Satta and Olmeo, 1980). Aspirin alone caused no significant change in any of the variables measured. In the unpaired study, frusemide greatly increased urine flow rate and sodium and osmolar clearances and these effects were antagonised by aspirin. However, these findings were not confirmed by the paired studies and surprisingly the higher dose of aspirin had less inhibitory effects. Overall there was no significant difference in the effect of frusemide in the presence or absence of aspirin in the same subjects. The blunting of the diuretic effect of frusemide by acetylsalicylic acid was thought to be due to inhibition of proximal tubular secretion rather than inhibition of prostaglandin synthesis.

SUMMARY

Acetylsalicylic acid is a weak organic acid with pKa 3.5 and its gastrointestinal absorption occurs predominately by passive infusion of nonionised molecules across the mucosal membrane.

Aspirin and other salicylates can inhibit the enzymatic production of prostaglandins.

Acetylsalicylic acid is rapidly hydrolysed to salicylic acid and the latter is conjugated with glycine and glucuronide with a limited capacity to form salicyluric acid, salicyl phenyl and salicyl acyl glucuronides respectively. A small amount of salicylic acid is hydroxylated /

hydroxylated to give gentisic acid and two other hydroxy metabolites. Diflunisal is a new salicylic acid derivative with longer duration of action which is conjugated with glucuronic acid.

Salicylates in therapeutic doses have pharmacological and sometimes toxic effects on most organs of the body. In overdosage they cause tinnitus, deafness, sweating, hyperventilation, respiratory alkalosis and metabolic acidosis. Salicylate metabolism and elimination are dose-dependent. The renal excretion of salicylate involves glomerular filtration, tubular conjugation, active tubular secretion and passive tubular reabsorption.

The renal excretion and clearance of salicylic acid is pH dependent, and thus administration of sodium bicarbonate may enhance elimination. The urine flow rate also affects salicylate elimination, and forced alkaline diuresis is often employed for the treatment of salicylate poisoning.

SECTION I

CHAPTER 2

PURPOSE AND OUTLINE OF THE STUDY

(a) Objectives

The author has been concerned with the management of poisoning for many years, first as physician in charge of the Poisoning Treatment Unit, Mashhad University, Iran, and subsequently as Research Fellow in the University Department of Therapeutics and Clinical Pharmacology and the Regional Poisoning Treatment Centre at the Royal Infirmary, Edinburgh. In spite of the different pattern of poisoning in Mashhad, analgesic overdosage is one of the commonest causes of admission to the Unit (second to hypnotics), and salicylate poisoning is the commonest analgesic overdosage (Balali-Mood and Salehi-Milani, 1979).

Acetylsalicylic acid is one of the cheapest, most easily available and most widely used drugs in the world. In the United Kingdom more than two million kg of aspirin is consumed every year; an average of about 2 tablets per week for every member of the population. In the United States of America, the average intake per head is about twice that of the United Kingdom (Bowman et al., 1980). In the United Kingdom, the number of aspirin tablets sold to the public increased from 3156 million in 1973 to 3450 million in 1976, whereas the corresponding sale of paracetamol was 2526 and 2872 million tablets respectively (Fryers, 1977).

Aspirin was for many years the most frequently taken poison and killer of children in the United States (Kaye, 1972). Although there has been a substantial reduction in the number of fatal poisonings /

poisonings in children under 5 years (61 deaths in 1968 compared with 26 in 1973) due to the use of safety packing and reduction in the unit dose of flavoured aspirin in the United States (Mofenson and Greensher, 1975; McQueen, 1977; Done, 1978), adult aspirin preparations pose the greater hazard (Done, 1978). Locket (1973) reported that the number of admissions for aspirin poisoning in the United Kingdom had increased steadily from 3800 in 1960 to more than 13,000 in 1973. Of the 16,000 children admitted to hospital for accidental poisoning in Great Britain each year, about 5,000 were due to the especially flavoured "Junior" aspirins (Sweetnam, 1974; Legg, 1974).

The number of patients admitted to hospital for salicylate poisoning between 1972 and 1974 in New Zealand had remained about the same (around 300 cases per year), but the number of deaths was higher in 1974 (3) than in 1972 (1) (McQueen, 1977). Since 1967 between 10 and 20 per cent of all admissions to the Regional Poisoning Treatment Centre, Royal Infirmary, Edinburgh, have been due to salicylate poisoning (Proudfoot and Park, 1978).

Poisoning with diflunisal is still not common and to date only one brief report of overdosage has been published (Upadhyay and Gupta, 1978). Forced alkaline diuresis has been employed routinely for the treatment of moderate to severe salicylate poisoning whereas haemodialysis and column haemoperfusion are reserved for very severe cases (Ferguson and Boutros, 1970).

Forced alkaline diuresis has been recommended for the treatment of diflunisal overdosage (ABPI Data Sheet Compendium 1979-1980) but the effects on its removal were unknown. In one report, it appeared to have little beneficial effect, although no measurements were made (Upadhyay and Gupta, 1978).

Different /

Different regimes of forced alkaline diuresis in salicylate poisoning have been used by many workers, but the effects of this treatment on salicylate distribution, metabolism and elimination have not been investigated in detail. The assays used to estimate the drug in overdose patients responded only to salicylic acid and did not measure metabolites and unchanged acetylsalicylic acid. A simple, sensitive and specific high performance liquid chromatographic assay was therefore developed to estimate acetylsalicylic acid and its metabolites in plasma and urine.

The present study was undertaken to investigate the causes of the following problems encountered with salicylate poisoning.

1. The increase in plasma salicylate concentrations observed despite gastric lavage in some patients with aspirin poisoning. This could be due to delayed absorption or slow hydrolysis of acetylsalicylic acid to salicylic acid after absorption.

2. Although plasma salicylate concentrations usually decline with clinical improvement after forced alkaline diuresis, the amount of salicylate recovered in the urine is not nearly enough to account for the fall observed. This spurious reduction in plasma salicylate could be due to fluid retention and increased volume of distribution of salicylate.

3. Effects of frusemide combined with forced alkaline diuresis on salicylate distribution and elimination following overdosage.

4. The role of protein binding of salicylic acid and its metabolites in salicylate poisoning.

5. Failure to adequately raise the urine pH in some patients.

6. /

6. Induction of hypokalaemia, hypocalcaemia, hypophosphataemia, hypomagnesaemia and other biochemical abnormalities.

7. Effects of prostaglandin-mediated effects of salicylate on the kidney (e.g. antidiuretic effect).

8. Haematological changes in aspirin poisoning.

9. Frequency of clinical features of salicylate poisoning and the effects of forced alkaline diuresis on clinical manifestations.

10. Effects of forced alkaline diuresis on diflunisal elimination.

(b) Outline of the study

1. Laboratory investigations

Sensitive, specific and reproducible high performance liquid chromatographic methods were developed for the estimation of acetylsalicylic, salicylic and salicyluric acids and diflunisal in plasma and urine. Salicylate protein binding was investigated in-vitro and in-vivo and compared with in-vitro albumin binding using an ultrafiltration technique. Solutions used for forced alkaline diuresis and forced diuresis were titrated against acid and base.

2. Healthy volunteers

The effects of changes in urine pH and flow rate on the elimination of acetylsalicylic acid and its metabolites and diflunisal were studied in healthy volunteers as the basis for the overdose study.

3. /

3. Overdose patients

The relative importance of urine flow rate and pH on salicylate elimination following overdosage was investigated in four groups of patients who received either forced diuresis, alkali alone, forced alkaline diuresis or forced alkaline diuresis with frusemide. Another group of patients with mild to moderate salicylate poisoning received no specific treatment as the controls. Clinical features, biochemical and haematological abnormalities were also studied.

SECTION II

ANALYTICAL METHODS FOR THE ESTIMATION OF ACETYLSALICYLIC ACID AND ITS METABOLITES AND DIFLUNISAL IN PLASMA AND URINE. MEASUREMENT OF SALICYLATE PROTEIN BINDING.

SECTION II

Chapter 1.

ESTIMATION OF ACETYLSALICYLIC ACID AND ITS METABOLITES IN PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

(a) - Review of analytical methods

Several methods have been reported for the estimation of salicylate including colorimetry, fluorimetry, ultra-violet spectrophotometry, gas liquid chromatography, liquid chromatography and high performance liquid chromatography.

A simple colorimetric method for routine estimation of plasma salicylic acid concentrations with the usual therapeutic doses was reported by Brodie, Udenfriend and Coburn (1944). This involved a separation of salicylic acid from plasma by extraction into ethylene chloride and its return to an aqueous phase as the coloured iron complex. Smith (1951) described another colorimetric method for the quantitation of acetylsalicylic and salicylic acids (with and without hydrolysis) using an aqueous solution of the Folin-Ciocalteu phenol reagent following two phases of acid and alkaline extractions. This assay was simplified by employing only one plasma sample for both salicylates, but two calibration curves at the end which resulted in more accurate estimation of acetylsalicylic acid and better recovery than the Smith method (Muni, Leeling, Helms, Johnson, Bare and Phillips, 1978) Trinder (1954) developed a rapid colorimetric assay /

assay for the determination of salicylate in biological fluids based on a reagent containing ferric nitrate, mercuric chloride and hydrochloric acid, which precipitates the proteins and simultaneously reacts with salicylic acid to give a purple colour.

Sensitive fluorimetric methods for the specific estimation of salicylic acid and each of its principal metabolites in plasma and urine using spectrophotofluorometry were described by Schachter and Manis (1958). With these methods, the salicyl conjugates were detected and estimated in the plasma of normal subjects given a single oral dose of salicylate.

A simple ultraviolet spectrophotometric method was developed for the simultaneous determination of salicylic acid, acetylsalicylic acid, salicylamide, caffeine and phenacetin in tablets and powders at three different wavelengths and under three different conditions of acid and base content (Clayton and Thiers, 1966).

Rowland and Riegelman (1967) described a specific and sensitive method for estimation of acetylsalicylic and salicylic acids in plasma. These compounds were extracted from acidified plasma with ether. Acetylsalicylic acid was measured by gas liquid chromatography following derivatisation and salicylic acid was measured spectrophotofluorometrically. A rapid, sensitive gas liquid chromatographic method for the simultaneous analysis of acetylsalicylic and salicylic acids in admixtures and in single component aspirin tablets was reported by Watson, Crescuolo and Matsui (1971). This method was modified to quantitate acetylsalicylic and salicylic acids in human plasma as well (Tam, Au and Abbott, 1979). Other gas liquid chromatographic methods for the estimation of acetylsalicylic and salicylic acids were /

were also reported (Thomas, Solomonraj and Coldwell, 1973; Walter, Biggs and Coutts, 1974).

Conditions for the routine separation and quantitation of aspirin, caffeine, 4-hydroxyacetanilide, 4-ethoxyacetanilide and salicylamide by liquid chromatography were described by Stevenson and Burtis (1971). An automated high-pressure liquid chromatographic method for the separation and determination of aspirin, phenacetin and caffeine in pharmaceutical dosage forms was reported (Ascione and Chrekian, 1975). Other high-pressure liquid chromatographic assays for the estimation of aspirin and salicylic acid in different dosage forms were also reported (Das Gupta, 1980a; Das Gupta, 1980b; Kirchhoefer and Juhl, 1980; Kirchhoefer, 1980).

A simple and rapid high-pressure liquid chromatographic method was developed to estimate acetylsalicylic, salicylic and salicyluric acids simultaneously in plasma. The procedure involved the solvent extraction of these compounds from acidified plasma with a benzene ethyl acetate mixture using phthalic acid used as internal standard. The mobile phase was 30% (V/V) acetonitrile in diluted phosphoric acid with a flow rate of 1 ml/min and ultraviolet detection at 237 nm (Peng, Gadalla, Smith, Peng and Chiou, 1978). Two other similar high-pressure liquid chromatographic methods for the estimation of acetylsalicylic and salicylic acids in plasma were also reported (Ali, McDonald, Thiessen and Coates, 1980; Lo and Bye, 1980). Another high-pressure liquid chromatographic assay was developed to determine salicylic, salicyluric and gentisic acids in mouse and rat blood, urine and faeces and rat embryos. The procedure involved aqueous dilution of the biological sample, addition of methanol - 1% acetic acid in water (40 : 60), centrifugation and injection of the supernatant onto the column /

column (Maulding and Young, 1980). A simultaneous liquid chromatographic quantitation of salicylic, salicyluric and gentisic acid in plasma using ultra-violet detection at 313 nm and two solvents (5% acetic acid in water and pure methanol) at 2.6 ml/min flow rate, was described (Cham, Johns, Bochner, Imhoff and Rowland, 1979). They used o-methoxybenzoic acid as internal standard and acetonitrile as protein precipitant with no solvent extraction. Sensitivity was 20 µg/L with linear response and complete recovery. Another simultaneous determination of salicylic acid, indomethacin and its major metabolites in serum and urine by reversed-phase liquid chromatography was reported (Terweij-Groen, Heemstra and Kraak, 1980).

In conclusion, the colorimetric and fluorometric determinations of acetylsalicylic and salicylic acids are not specific and measure acetylsalicylic acid only by difference after hydrolysis to salicylic acid. The differential ultra-violet spectrophotometry of the two salicylates based on the pH-dependent shift in their absorbance may require correction because of overlap of their absorption spectra. Furthermore, these methods may yield high and variable blank values. Gas liquid chromatographic assays are specific and sensitive and permit the measurement of both compounds simultaneously, but extraction and chemical derivatisation are needed, which are time-consuming and can be complicated by hydrolysis of acetylsalicylic acid to salicylic acid and multiple product formation. High performance liquid chromatographic methods which involved solvent extraction are again time-consuming and complicated by the acetylsalicylic acid hydrolysis, but those without solvent extraction that measure acetylsalicylic, salicylic, salicyluric and gentisic acids simultaneously are appropriate.

Acetylsalicylic acid hydrolysis

It is well known that acetylsalicylic acid is rapidly hydrolysed in aqueous solution and its hydrolysis accelerated by esterases found in the blood or plasma (Lester et al., 1946). The in vitro hydrolysis was found to obey pseudo-first-order kinetics with a mean half-life of 32 minutes for blood and 66 minutes for plasma at a concentration of 13 µg/ml. Excessive concentrations of acetylsalicylic acid might saturate the enzymes and cause the reaction to follow zero-order kinetics (Harris and Riegelman, 1967). It has been shown that the hydrolysis rate of aspirin is constant with minimum hydrolysis at pH 4 and maximum at pH 8 (Landecker, Wellington, Thomas and Piper, 1977; Dearden and George, 1979).

(b) Development of high performance liquid chromatographic methods for the simultaneous determination of acetylsalicylic acid and its metabolites in plasma and urine

(i) Materials

Acetylsalicylic acid and ethyl acetate were obtained from May and Baker Ltd., salicyluric acid, gentisic acid and benzoic acid from Sigma Chemical Company, salicylic acid, acet-p-toluidide, isopropanol, sodium acetate, monopotassium phosphate, disodium phosphate, sodium chloride, potassium fluoride and potassium nitrate from BDH, methanol from James Burroughs Ltd., glacial acetic acid from Koch-Light Laboratories Ltd., perchloric acid from Fisons Scientific Apparatus and β-glucuronidase from Sigma Chemical Co.

(ii) Instrumentation

The high performance liquid chromatographic system consisted of /

of a Pye Unicam LC3 UV detector, set up at 243 nm (although the maximum UV-absorbance for acetylsalicylic, salicylic and salicyluric acids was 220, 230 and 239 nm respectively, because of ethyl acetate in the mobile phase 243 nm was chosen as the wavelength), an Orlita DMP AE 10.4 pump, a Bryans Model 28000 recorder and a Shandon type column and septum injector. The column was 150 x 4.5 mm i.d. internally polished, stainless steel, slurry packed with Hypersil ODS, C18 - TMS (Shandon), 5 μ particle size.

(iii) Plasma assay

Chromatographic conditions

The mobile phase consisted of 0.02 M potassium nitrate in 2% acetic acid, isopropanol and ethyl acetate (100:12:4) and was degassed under reduced pressure before use. The flow rate was 1.2 ml/min with a working pump pressure of 1200 psi. Detector sensitivity was set at 0.08 absorbance units full scale and changed to 0.32 after the acet-p-toluidide peak had eluted.

Preparation of standard solutions for plasma assay

Aqueous solutions of salicylic acid (2 mg/ml), salicyluric acid (1 mg/ml) and acet-p-toluidide (500 μg/ml) as internal standard for acetylsalicylic and salicyluric acids were prepared and kept under refrigeration (4°C). The pH of the solutions were 3.0, 3.3 and 4.9 respectively and did not change during the study. These standard solutions were stable and used for up to six months. Benzoic acid was used as the second internal standard, for salicylic acid. Acetylsalicylic and benzoic acid standard solutions (500 μg/ml) were

were always made up freshly before use. The internal standard solution contained 100 µg/ml benzoic acid and 5 µg/ml acet-p-toluidide in 6% perchloric acid and was used as the plasma protein precipitant.

Plasma standards were prepared by spiking blank plasma from five healthy volunteers with acetylsalicylic acid (10-200 µg/ml), salicylic acid (50-1000 µg/ml) and salicyluric acid (0.5-10 µg/ml).

Collection and storage of plasma samples

The blood samples were collected into lithium heparin tubes (Searle Diagnostic, High Wycombe) containing 50 µl of 20% (W/V) potassium fluoride solution. Plasma was separated immediately and placed in glass tubes containing 50 µl of potassium fluoride solution and 50 µl of glacial acetic acid and stored at -20°C until analysis. Over 600 plasma samples from the healthy volunteers and patients with aspirin overdosage were collected, stored and assayed.

Procedure for plasma assay

To 100 µl of unknown plasma sample or the spiked plasma standard in a Dreyer tube was added 100 µl of the internal standard solution (5 µg/ml acet-p-toluidide and 100 µg/ml benzoic acid in 6% perchloric acid). After mixing, the sample was centrifuged at 2000 r.p.m. for 5 minutes and a 5-25 µl aliquot of the clear supernatant directly injected into the chromatograph.

Two plasma standards of acetylsalicylic, salicylic and salicyluric acids (100, 400, 5 µg/ml and 50, 200, 2.5 µg/ml respectively) were assayed with each set of unknown samples.

The plasma concentration of each compound was determined from the following /

following expression :-

$$M = \frac{R.M'}{R'}$$

where :

M = concentration of the compound in the plasma (μg/ml)

M'= concentration of the compound in the standard (μg/ml)

R = peak height response ratio of the compound to internal standard

R'= peak height response ratio of the standard to internal standard

The glucuronide conjugates were measured by the difference after hydrolysis as follows :

To 1 ml plasma was added 0.5 ml of 0.1M acetate buffer pH 5.0 and 0.1 ml β-glucuronidase 120,000 activity, and after mixing, incubated overnight at 37°C. The total salicylic and salicyluric acids were measured as above

Results of plasma assay

A typical chromatogram of plasma obtained from a patient with mild salicylism is shown in Figure 2.1. The retention times of salicyluric acid, acetylsalicylic acid, acet-p-toluidide, benzoic acid and salicylic acid were 3, 3.5, 5, 7 and 10 minutes respectively. No other interfering peaks were observed.

Validation of plasma assay

The calibration graphs of acetylsalicylic, salicylic and salicyluric acids were linear and passed through the origins (Figs. 2.2, 2.3 and 2.4 respectively). The precision and reproducibility for each compound are shown in Tables 2.1, 2.2 and 2.3 as the results of 5 replicate analyses of the spiked plasma. The overall recoveries (drug /

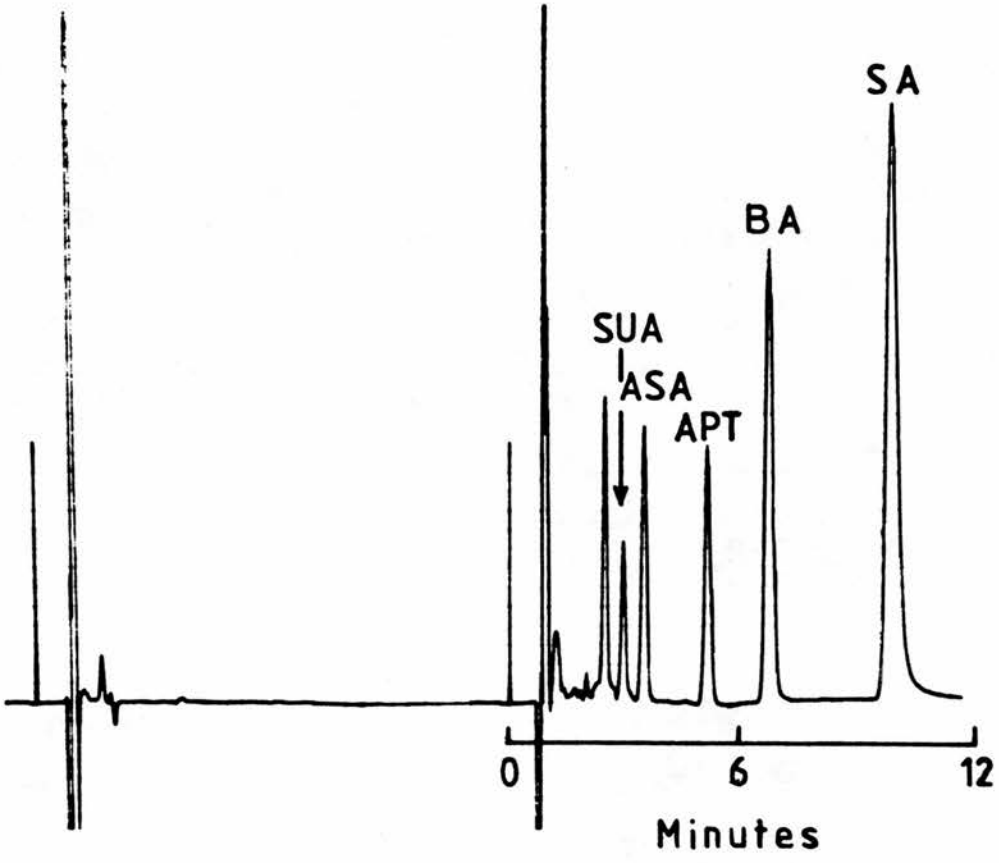

Figure 2.1. Chromatogram of blank plasma (left) and plasma from a patient with aspirin overdosage (right). SUA = salicyluric acid, ASA = acetylsalicylic acid, APT = acet-p-toluidide and BA = benzoic acid as internal standards, SA = salicylic acid.

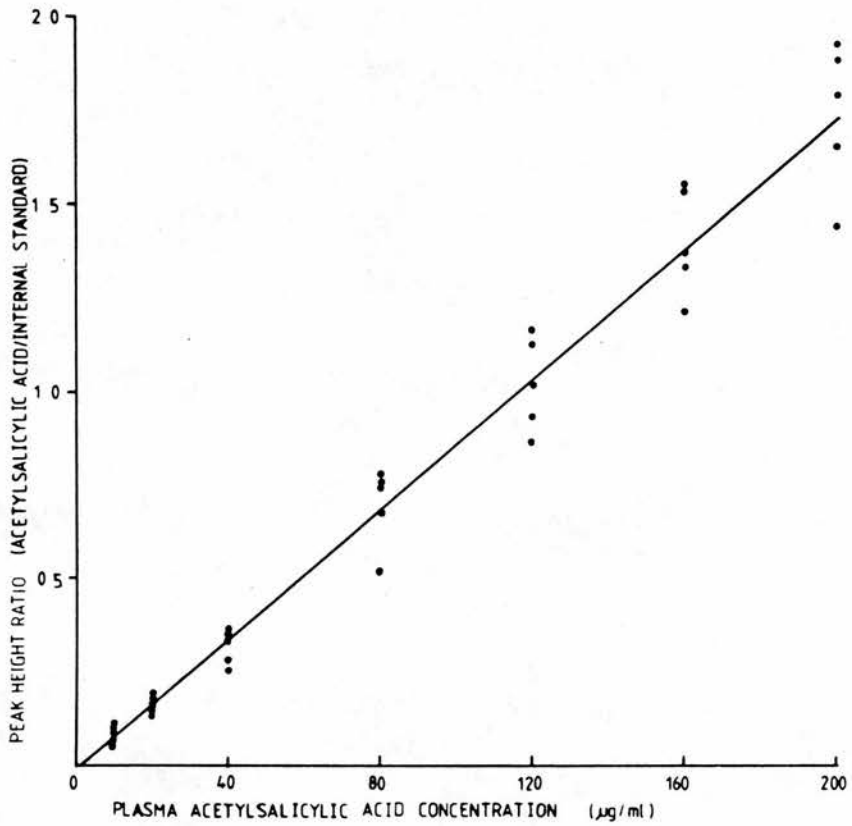

Figure 2.2. Calibration graph for the estimation of acetylsalicylic acid in plasma, using high performance liquid chromatography with acet-p-toluidide as internal standard.

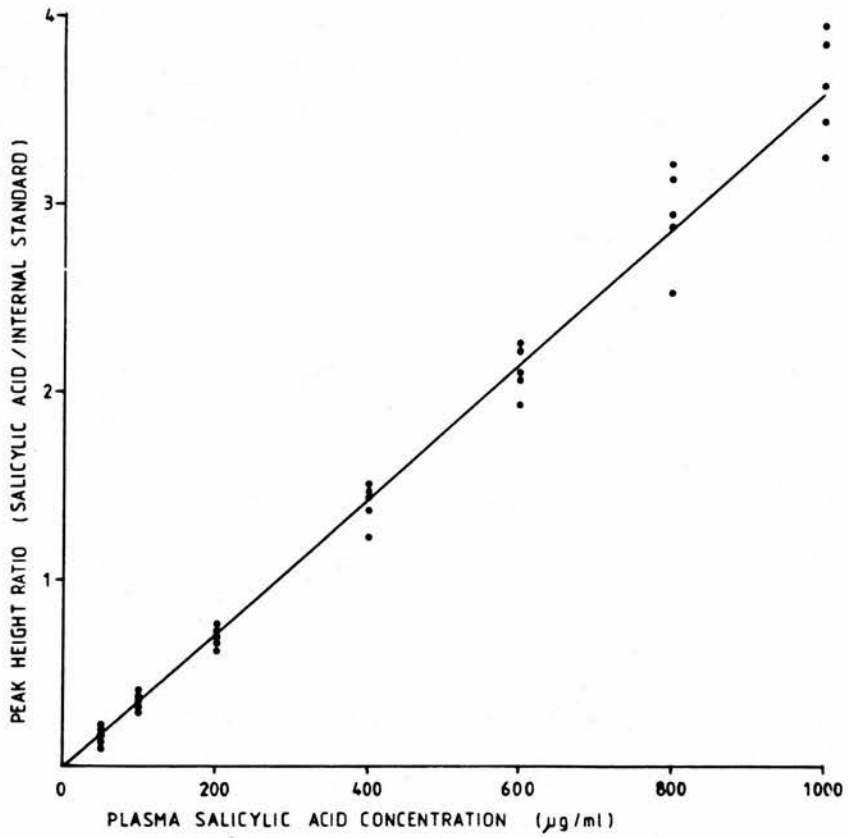

Figure 2.3. Calibration graph for the estimation of salicylic acid in plasma, using high-pressure liquid chromatography with benzoic acid as internal standard.

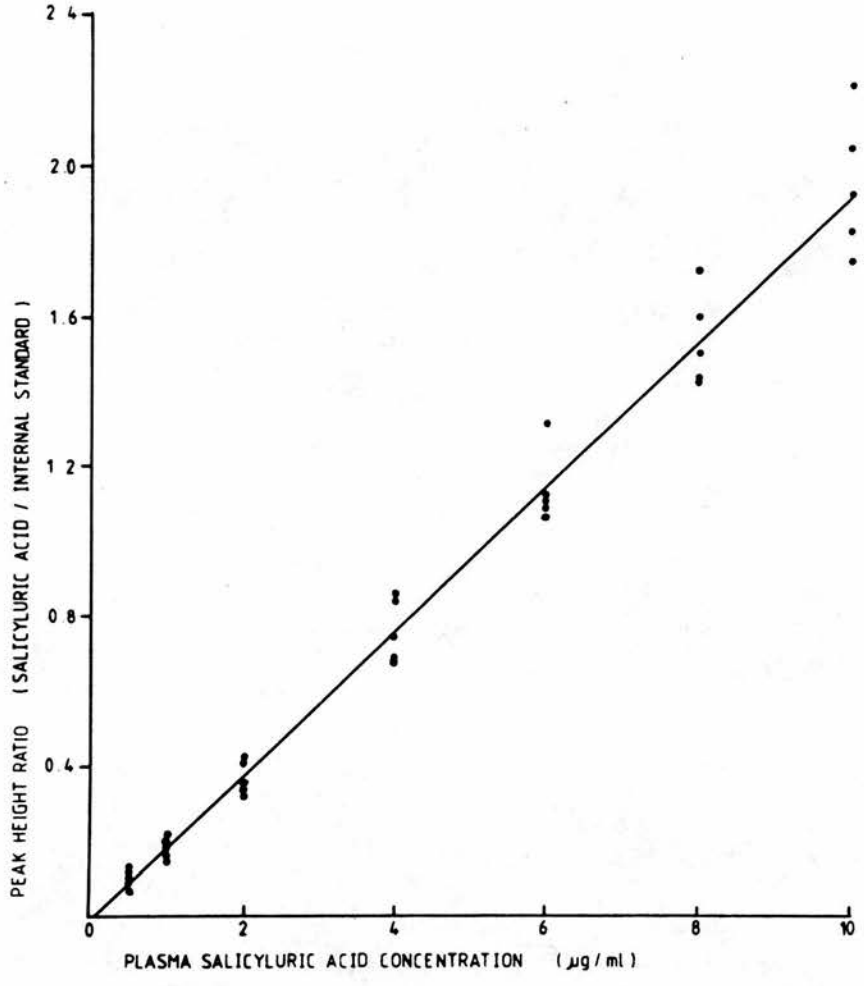

Figure 2.4. Calibration graph for the estimation of salicyluric acid in plasma using high-pressure liquid chromatography with acet-p-toluidide as internal standard.

TABLE 2.1. REPLICATE ANALYSES OF ACETYLSALICYLIC ACID IN PLASMA

Drug added (μg/ml)	Drug found (μg/ml) Individual values					Mean	S.D.	Coefficient of variation %
	Run 1	Run 2	Run 3	Run 4	Run 5			
10	9.47	10.2	12.6	11.8	9.5	10.71	1.42	13.26
20	19.7	20.0	22.1	22.2	20.2	20.84	1.21	5.80
40	38.9	39.2	38.1	38.8	40.4	39.08	0.84	2.15
80	77.9	78.9	74.2	74.4	79.6	77.0	2.54	3.30
120	117.8	121.6	119.6	115.9	120.0	118.98	2.20	1.85
160	163.1	162.3	166.3	163.4	160.4	163.1	2.14	1.31
200	202.0	197.7	197.1	201.4	199.7	199.58	2.17	1.09

TABLE 2.2. REPLICATE ANALYSES OF SALICYLIC ACID IN PLASMA

Drug added (μg/ml)	Drug found (μg/ml) Individual values					Mean	S.D.	Coefficient of variation %
	Run 1	Run 2	Run 3	Run 4	Run 5			
50	45.5	51.3	56.7	50.6	49.8	50.8	4.00	7.88
100	94.2	98.1	105.2	102.6	99.6	99.9	4.22	4.22
200	191.6	191.5	194.7	208.2	202.1	197.6	7.32	3.70
400	383.3	389.7	389.0	394.5	401.4	391.6	6.78	1.73
600	600.0	600.0	588.0	578.0	579.9	589.2	10.6	1.79
800	788.5	796.2	828.7	817.6	794.4	805.1	17.20	2.14
1000	1018	958.9	987.2	988.5	1005	991.5	22.22	2.24

TABLE 2.3. REPLICATE ANALYSES OF SALICYLURIC ACID IN PLASMA

Drug added (μg/ml)	Drug found (μg/ml) Individual values					Mean	S.D.	Coefficient of variation %
	Run 1	Run 2	Run 3	Run 4	Run 5			
0.5	0.49	0.52	0.54	0.59	0.54	0.54	0.04	7.40
1.0	0.85	0.98	1.01	1.06	1.04	0.99	0.08	8.08
2.0	2.04	1.89	1.93	1.87	2.04	1.95	0.08	4.10
4.0	3.98	3.82	4.07	3.80	4.19	3.97	0.17	4.30
6.0	6.06	6.09	5.95	6.16	5.44	5.94	0.30	5.05
8.0	7.96	8.20	7.72	8.15	7.99	8.00	0.20	2.5
10.0	10.2	9.97	10.2	9.86	10.2	10.09	0.16	1.59

drug found) of acetylsalicylic, salicylic and salicyluric acids for the spiked plasma at the concentrations used were 100.9 ± 6.5, 99.6 ± 3.8 and 100.4 ± 5.5 (mean ± SD) % respectively. The accuracy of the assay was determined by assaying two national quality control plasma samples of salicylic acid on two occasions. Results of 400 µg/ml and 437 µg/ml for the controls of 400 µg/ml and 433 µg/ml respectively were obtained on one occasion and 415 µg/ml and 410 µg/ml for the controls of 410 µg/ml and 410 µg/ml on another. The limits of detection were 0.2 µg/ml, 0.5 µg/ml and 2 µg/ml for salicyluric, acetylsalicylic and salicylic acids respectively.

Stability

A total of 198 plasma samples of acetylsalicylic, salicylic and salicyluric acids were prepared (100 µl of each in a Dreyer tube) and stored under different conditions (Table 2.4). The samples were assayed on the day of preparation, weekly up to 8 weeks and then at the 11th and 23rd weeks.

Salicyluric acid (5 µg/ml) was stable at -20°C and 4°C, with or without the other compounds for 23 weeks. The loss of salicylic acid (200 µg/ml) with potassium fluoride and acetic acid was 8.5% at 4°C by the 11th week and 19% by the 23rd week, whereas without the preservatives the losses were 20% and 30% respectively at -20°C. The concentrations of salicylic acid stored with acetylsalicylic and salicyluric acids without preservatives at -20°C increased up to 22%, but with the preservatives at -20°C did not change up to the 11th week and increased only 8% by the 23rd week. These increases were due to acetylsalicylic acid hydrolysis.

Acetylsalicylic /

TABLE 2.4. STABILITY TESTS OF ACETYLSALICYLIC, SALICYLIC AND SALICYLURIC ACIDS IN HUMAN PLASMA

Sample No.	Sample Contents	% remaining			
		Week 1	Week 8	Week 11	Week 23
1	50 µg/ml acetylsalicylic acid* at 4°C	0	0	0	0
2	200 µg/ml salicylic acid* at 4°C	100	93	91	81
3	5 µg/ml salicyluric acid* at 4°C	100	100	100	100
4	Samples) acetylsalicylic acid* at 4°C	83	8	7	0
5	1, 2 & 3) salicylic acid* at 4°C	100	117	115	107
6	combined) salicyluric acid* at 4°C	100	100	100	100
7	50 µg/ml acetylsalicylic acid* at -20°C	100	86	80	79
8	200 µg/ml salicylic acid* at -20°C	100	104	103	88
9	5 µg/ml salicyluric acid* at -20°C	100	100	100	94
10	Samples) acetylsalicylic acid* at -20°C	100	80	80	56
11	1, 2 & 3) salicylic acid* at -20°C	100	100	100	108
12	combined) salicyluric acid* at -20°C	100	100	100	104
13	50 µg/ml acetylsalicylic acid** at -20°C	0	0	0	0
14	200 µg/ml salicylic acid** at -20°C	100	100	80	70
15	5 µg/ml salicyluric acid** at -20°C	100	100	100	100
16	Samples) acetylsalicylic acid** at -20°C	0	0	0	0
17	1, 2 & 3) salicylic acid** at -20°C	122	117	116	111
18	combined) salicyluric acid** at -20°C	100	100	100	100

* with 2 µg/ml potassium fluoride in distilled water and 2 µl/ml glacial acetic acid in distilled water.
** without preservative

Acetylsalicylic acid (50 μg/ml) with the preservatives at 4°C or without at -20°C was not stable and hydrolysis was complete after 7 days. However, with salicylic and salicyluric acids and the preservatives it was stable up to 4 weeks at -20°C.

(iv) Urine assay

Chromatographic conditions

The mobile phase consisted of 0.02 M potassium nitrate in 2% acetic acid and isopropanol (100:18), and was degassed under reduced pressure prior to use. The flow rate was 1 ml/min with the working pump pressure of 1000 p.s.i.

Detector sensitivity was usually set at 0.16 absorbance units full scale, but appropriate changes were made for higher or lower concentrations. A 2 to 10 fold dilution of the sample prior to injection was necessary with samples containing more than 400 μg/ml.

Preparation of standard solutions

The aqueous standard solutions of acetylsalicylic, salicylic and salicyluric acids and acet-p-toluidide were prepared as described for the plasma assay. Acet-p-toluidide (50 μg/ml) was the only internal standard used.

Urine standard solutions were prepared by spiking blank urine from five different healthy volunteers with salicylic acid (50 - 400 μg/ml) and salicyluric acid (25-200 μg/ml).

Collection and storage of urine samples /

Collection and storage of urine samples

For the measurement of pH and for biochemical investigations, 20 ml aliquots of each urine sample were placed into Universal containers and the remainder collected in 1 or 2 litre plastic bottles containing 250 µl or 500 µl of 20% potassium fluoride solution and 250 µl or 500 µl of glacial acetic acid.

The pH (using a pH meter, Radiometer Model pH M62) and volume of each sample were measured immediately and 10-15 ml aliquots were stored at -20°C.

Procedure for urine assay

To 0.5 ml of urine or standard in a disposable polypropylene tube (Lukhams LP3), was added 0.5 ml of a solution containing 50 µg/ml acet-p-toluidide as the internal standard and 100 µl of 6% perchloric acid. After mixing and centrifugation 5-25 µl of the clear supernatant was directly injected into the chromatograph.

Perchloric acid was added to reduce the pH and to precipitate proteins.

At least three urine standards containing salicylic acid (50-400 µg/ml), salicyluric acid (25-200 µg/ml) and acetylsalicylic acid (10-200 µg/ml) were assayed with each set of unknown samples. The concentrations of each compound were determined using the standard calibration graphs.

Gentisic acid was also measured, but because of interfering peaks in some overdose patients and because of its unknown toxicological significance (urinary recovery of about 1% of the total dose) it was excluded in routine analyses.

Salicylic /

Salicylic and salicyluric acid glucuronide conjugates in urine were measured by the difference after hydrolysis as follows :
To 0.5 ml of urine was added 0.5 ml of 0.1M acetate buffer pH5 and 0.1 ml β-glucuronidase and the mixture was incubated at 37°C overnight. Total salicylic and salicyluric acid concentrations in urine were measured as described above.

Results of urine assay

A typical chromatogram of urine obtained from a healthy volunteer 3 hours after oral administration of 20 mg/kg aspirin is shown in Figure 2.5. The retention times of gentisic, salicyluric and acetylsalicylic acids, acet-p-toluidide (internal standard) and salicylic acid were 3.3, 3.7, 5, 8 and 12 minutes respectively. No other interfering peaks were observed except with gentisic acid in some overdose patients.

The effects of pH on the precision and reproducibility of the assay were studied in urine and water standard solutions containing 200 μg/ml of salicylic acid. The mean peak height ratios of salicylic acid to internal standard did not change in the water standard with increasing pH, but the peak height ratios in the urine standards decreased from 0.73 at pH 1.9 to 0.22 at pH 7.9. The explanation for this finding is unknown.

Validation of urine assay

The standard calibration graphs were linear and passed through the origin (Figs. 2.6 and 2.7).The precision and reproducibility of the assay for each compound are shown in Tables 2.5 and 2.6 as the results /

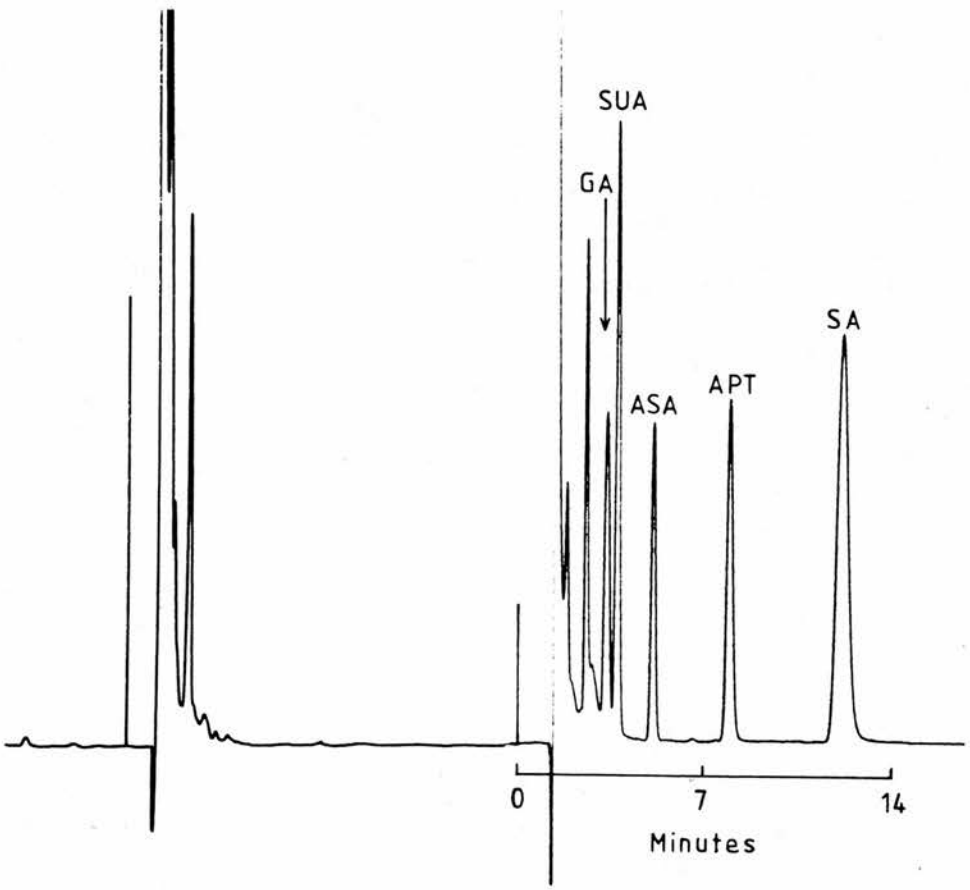

Figure 2.5. A typical chromatogram of urine obtained from a healthy volunteer at time 0 (left) and 3 hours after oral administration of 20 mg/kg aspirin (right). GA = gentisic acid, SUA = salicyluric acid, ASA = acetylsalicylic acid, APT = acet-p-toluidide as internal standard, SA = salicylic acid.

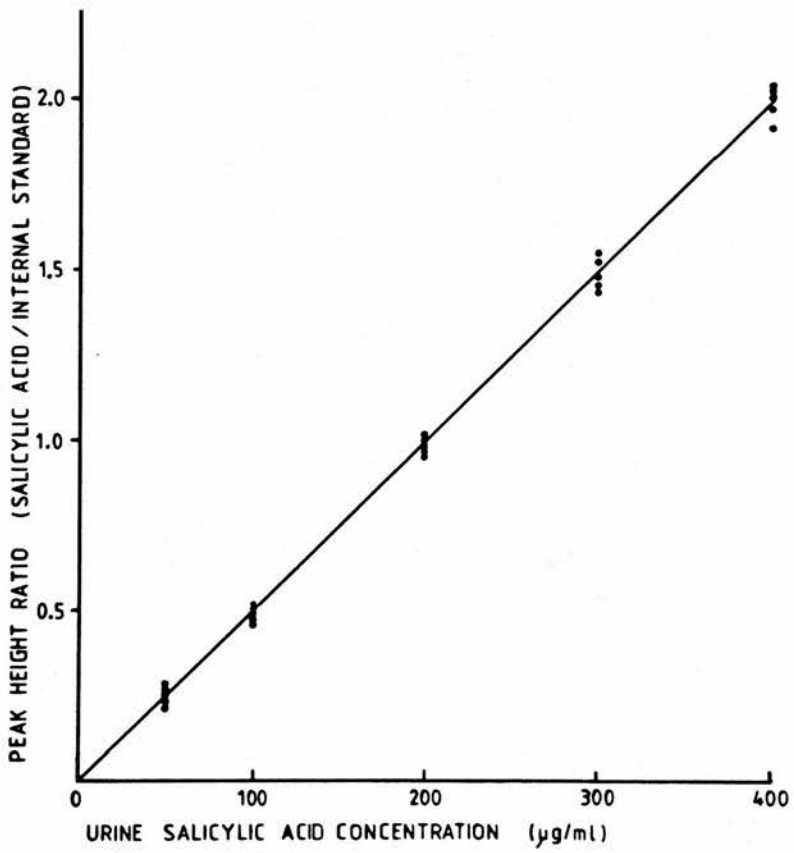

Figure 2.6. Calibration graph for the estimation of salicylic acid in urine, using high performance liquid chromatography with acet-p-toluidide as internal standard.

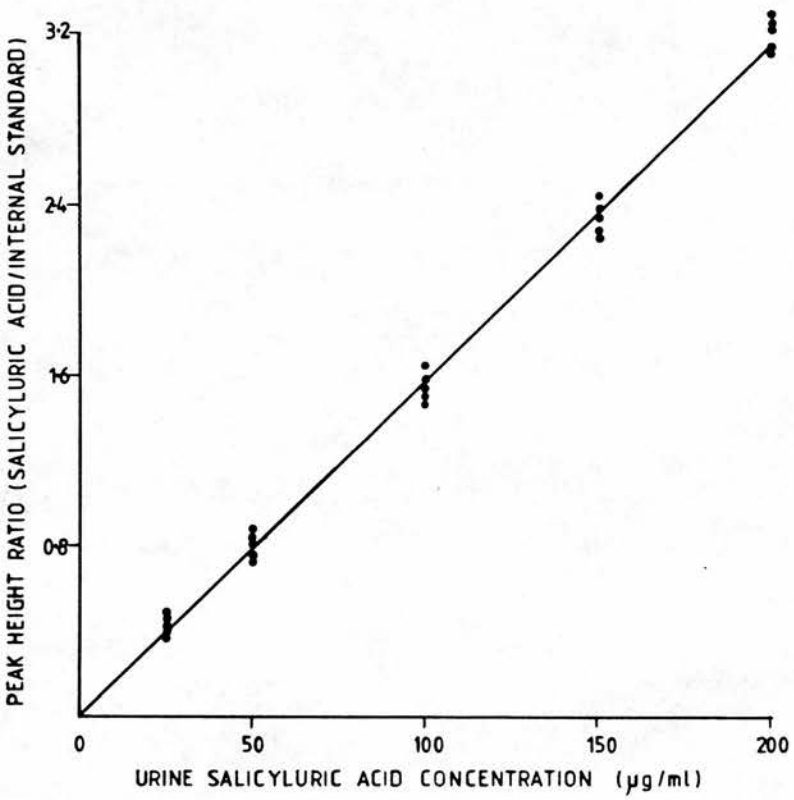

Figure 2.7. Calibration graph for the estimation of salicyluric acid in urine, using high performance liquid chromatography with acet-p-toluidide as internal standard.

TABLE 2.5. REPLICATE ANALYSES OF SALICYLIC ACID IN URINE

Drug added (µg/ml)	Drug found (µg/ml) Individual values					Mean	S.D.	Coefficient of variation %
	Run 1	Run 2	Run 3	Run 4	Run 5			
50	51.9	48.6	48.8	55.3	49.6	50.8	2.82	5.54
100	99.4	97.6	99.2	98.6	101.8	99.3	1.55	1.56
200	198.2	204.6	204.0	195.0	197.9	199.9	4.18	2.09
300	299.0	301.8	298.6	295.4	300.2	299.0	2.37	0.79
400	401.6	397.1	399.4	405.6	400.5	400.8	3.14	0.78

TABLE 2.6. REPLICATE ANALYSES OF SALICYLURIC ACID IN URINE

Drug added (μg/ml)	Drug found (μg/ml) Individual values					Mean	S.D.	(Coefficient of variation) %
	Run 1	Run 2	Run 3	Run 4	Run 5			
25	28.9	24.5	25.4	28.0	23.5	26.1	2.30	8.83
50	49.5	51.5	51.2	49.5	52.0	50.7	1.17	2.30
100	97.5	99.2	97.0	96.3	100.2	98.4	1.61	1.64
150	143.1	148.8	151.2	148.3	149.0	148.1	3.00	2.02
200	206.1	201.0	200.3	202.9	200.4	202.1	2.45	1.21

results of 5 replicate analyses. The overall mean recoveries (drug found) of salicylic and salicyluric acids for the concentration ranges of spiked urine were 99.9 ± 0.4 and 100.8 ± 2.4% (S.D.) respectively. The limits of detection were 1 and 5 μg/ml for salicyluric and salicylic acids respectively.

A preliminary report of this method with the high performance liquid chromatographic assays of other analgesic drugs has already been published (Prescott, King, Brown, Balali and Adriaenssens, 1979).

(c) Comparison of colorimetric and high performance liquid chromatographic methods

A modification of the method of Trinder (1954) was used as a ward side room assay for the rapid estimation of plasma salicylate in patients with aspirin overdosage.

Materials : Ferric nitrate, 0.038 M nitric acid and a colorimeter (Corning Model 252).

Method : Two solutions were prepared as the blank (0.038 M nitric acid) and reagent (11 g ferric nitrate diluted to 2 litres with the blank solution). The colorimeter was set up at zero using the blank and the absorbance after formation of the coloured iron-salicylate complex was read at 540 nm.

Results : In 39 samples from 19 patients with aspirin overdosage salicylate concentrations were measured by both methods (Fig. 2.8). The results with colorimetric assay were 11.4 ± 7.3% higher than with the high performance liquid chromatographic method.

(d) Summary and conclusions /

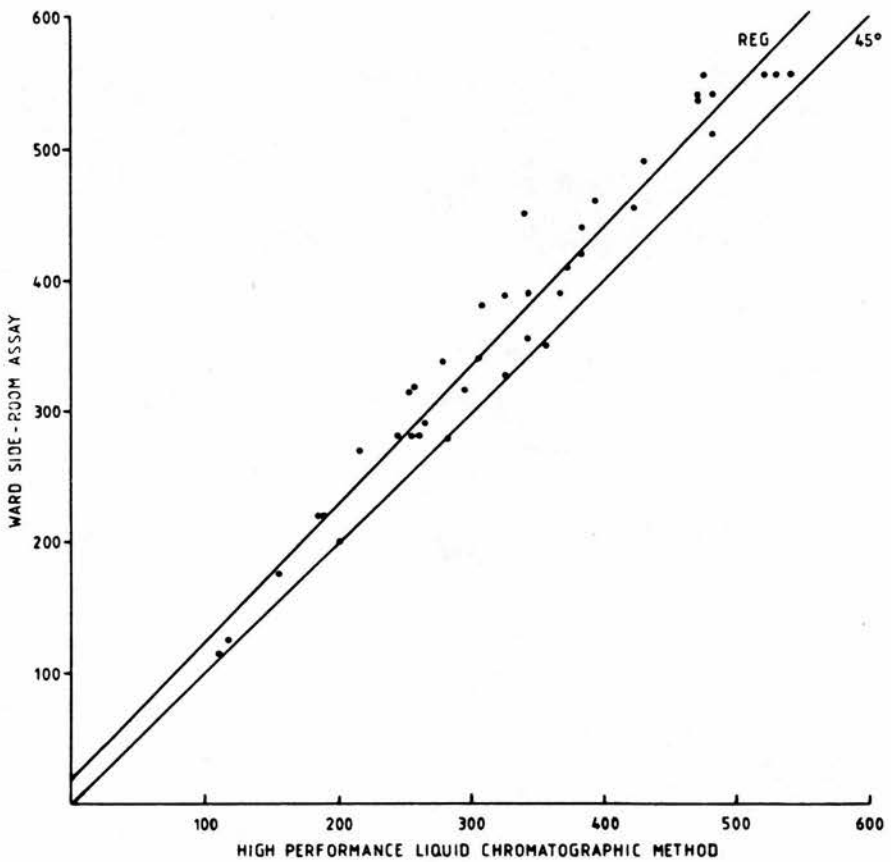

Figure 2.8. Comparison of the high performance liquid chromatographic method and ward side-room assay (modified Trinder's method) for the estimation of plasma salicylic acid concentrations.

(d) Summary and conclusions

Simple, specific, sensitive and reproducible high performance liquid chromatographic assays were developed for the simultaneous estimation of acetylsalicylic, salicylic and salicyluric acids in plasma and acetylsalicylic, salicylic, salicyluric and gentisic acids in urine. Acet-p-toluidide and benzoic acid were used as the internal standards for the plasma assay and acet-p-toluidide for the urine assay. Perchloric acid (6%) was used for the precipitation of proteins and following centrifugation the clear supernatant was injected directly into the chromatograph.

The standard calibration graphs for each compound were linear and passed through the origin. Good separation and reproducibility were obtained. The limits of sensitivity were 0.5 μg/ml, 1 μg/ml and 5 μg/ml for salicyluric, acetylsalicylic and salicylic acids respectively.

Rapid colorimetry (modified Trinder's method) was used as a ward side room assay for the rapid estimation of plasma salicylate from patients with aspirin overdosage. The results of this assay were an average of 11.4% higher than the concentrations measured by high performance liquid chromatography.

SECTION II

Chapter 2.

ESTIMATION OF DIFLUNISAL IN PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC AND FLUOROMETRIC METHODS

(a) Introduction

Gas chromatographic, radio-isotopic and fluorometric methods have been used for quantitation of diflunisal (Tocco, Breault, Zacchei, Steelman and Perrier, 1975), but all have disadvantages. The gas chromatographic assay includes extraction and evaporation stages and derivatisation, while the radio-isotopic and fluorometric methods are non-specific.

Since the present work was started, a high-performance liquid chromatographic method for the determination of diflunisal in plasma has been reported (Van Loenhout, Ketelaars, Gribnau, Van Ginneken and Tan, 1980). The diflunisal and naproxen (internal standard) are extracted from plasma with ether and n-hexane followed by evaporation to dryness. The diflunisal peak was broad and tailed, relative errors were 8.85% at low concentrations and the limit of detection was only 5 μg/ml. The present method does not require extraction. It can be completed in a fraction of the time, chromatography is more efficient and the results superior.

(b) Development of high performance liquid chromatographic methods for the estimation of diflunisal in plasma and urine

Materials /

Materials

Diflunisal was obtained from Thomas Morson Pharmaceuticals, Division of Merck Sharp & Dohme Ltd., Hoddesdon, Hertfordshire, and flufenamic acid (the internal standard) from Parke Davis & Co. (Usk Road, Pontypool, Gwent). All solvents and reagents were obtained commercially.

Preparation of standard solutions

5 mg of diflunisal was dissolved in 1.0 ml of methanol in a 100 ml volumetric flask and brought to volume with 1/15-molar(M) phosphate buffer (pH 7.2). Further dilutions were made with water or plasma to produce final concentrations of 1-10 and 10-100 µg/ml. Flufenamic acid (5 mg) was dissolved in 1.0 ml methanol in a 100 ml volumetric flask and made up to volume with 0.001 M sodium bicarbonate. The stock solutions were stored for 8 weeks at 4°C.

Standards were prepared by spiking blank urine or plasma from five healthy volunteers.

Chromatographic conditions

The high performance liquid chromatographic system consisted of a Pye LC3 UV detector set at 251 nm, an Orlita DMP AE 10.4 pump, a loop injector (Rheodyne Model 7120) and a recorder (Bryans Model 28000). The column was 150 X 4.5 mm i.d. internally polished stainless steel slurry packed with 5 µm Hypersil O.D.S. (Shandon).

The mobile phase was a mixture of 0.08 M potassium nitrate in 2% acetic acid, isopropanol and ethylacetate (55:25:20) which was degassed under reduced pressure prior to use.

Procedure for plasma assay /

Procedure for plasma assay

To 0.5 ml of plasma containing 10-100 μg/ml of diflunisal in a disposable polypropylene tube was added 0.5 ml of flufenamic acid solution (500 μg/ml) followed by 0.5 ml of acetone to precipitate the proteins. After mixing, the sample was centrifuged for 5 minutes and 25 μl aliquots of the clear supernatant directly injected into the chromatograph. For lower concentrations 50 μl of the flufenamic acid solution was used.

Plasma diflunisal concentrations of more than 100 μg/ml are diluted appropriately with normal saline.

The flow rate was 1.1 ml/min with the working pump pressure of 1600 p.s.i. Detector sensitivity was 0.08 absorbance units full scale.

Procedure for urine assay

To 1.0 ml of urine containing 1-10 μg/ml of diflunisal in a disposable polypropylene tube was added 100 μl flufenamic acid solution (500 μg/ml). After mixing, 25 μl was injected directly into the chromatograph.

Urine containing more than 10 μg/ml of diflunisal was diluted appropriately with distilled water.

The flow rate was 1.3 ml/min with the working pump pressure of 1800 p.s.i. Detector sensitivity was set at 0.02 absorbance units full scale.

Two standards of diflunisal (5 and 50 μg/ml in plasma) and (1 and 10 μg/ml in urine) were assayed with each set of unknown samples. Diflunisal concentrations were calculated from peak height ratios /

ratios as described for the assay of acetylsalicylic acid and metabolites (Chapter 1, Section II).

Results

Two typical chromatograms of plasma and urine obtained from a volunteer 3 hours after ingestion of 750 mg of diflunisal are illustrated in Figures 2.9. and 2.10.The retention time of diflunisal was about 6 minutes in both assays and samples could be injected every 9 minutes. No other interfering peaks were observed.

The standard calibration graphs for the plasma and urine assays were linear and passed through the origin (Figures 2.11 and 2.12). The precision and reproducibility of the plasma and urine assays are shown in Tables 2.7 and 2.8 respectively as the results of 5 replicate analyses of spiked samples. The overall recovery (drug found) of diflunisal was 100.4 ± 3.6 for plasma and 100.2 ± 5.3% for urine. The accuracy was determined by assaying two control plasma and urine samples containing 99.94, 10.95, 9.55 and 1.86 μg/ml of diflunisal respectively. The results of the assays were 98.93, 10.42, 9.95 and 1.75 μg/ml. The limits of detection were 1.0 μg/ml and 0.5 μg/ml for plasma and urine respectively.

The results of analyses in 17 plasma and 13 urine samples from 5 patients with diflunisal overdosage are given in Table 2.9.

Other considerations

Naproxen was initially chosen as the internal standard because it eluted before diflunisal with good separation. However, an interfering peak appeared in the urine of healthy volunteers following ingestion /

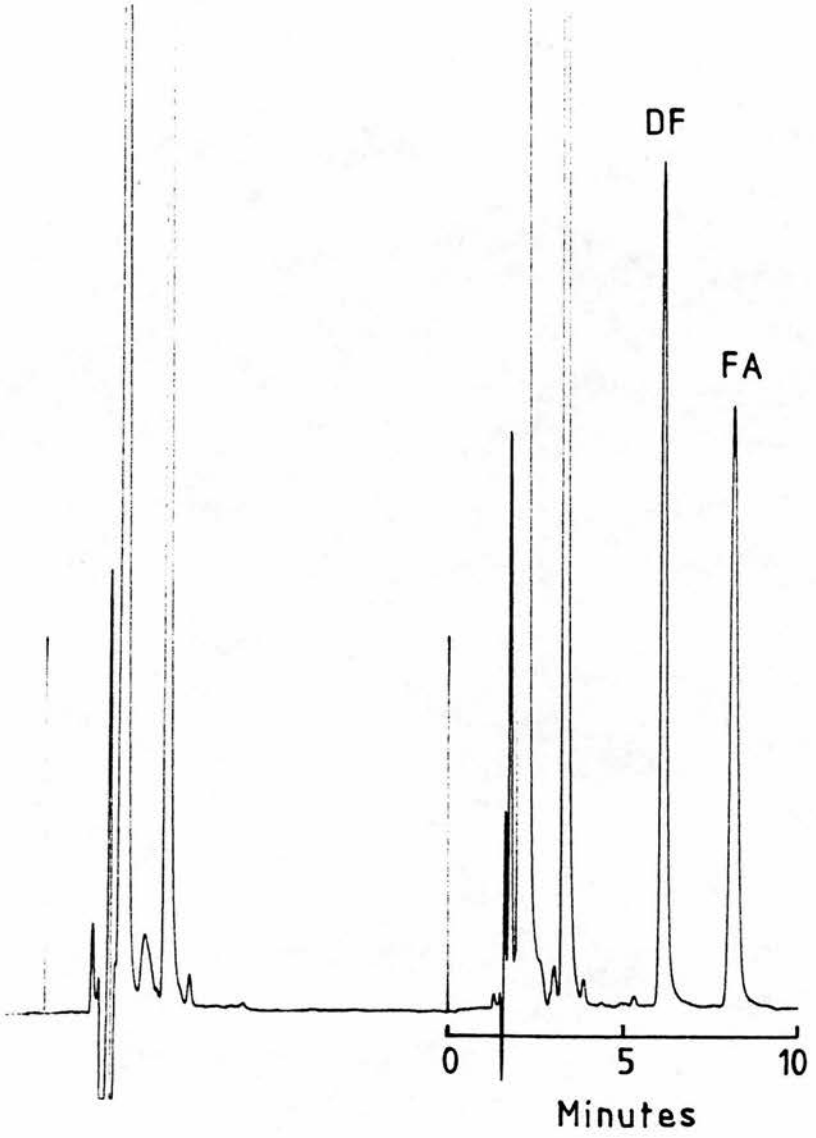

Figure 2.9. A typical chromatogram of plasma from a healthy volunteer at time 0 (left) and 3 hours after ingestion of 750 mg of diflunisal (right). DF = diflunisal, FA = flufenamic acid (internal standard).

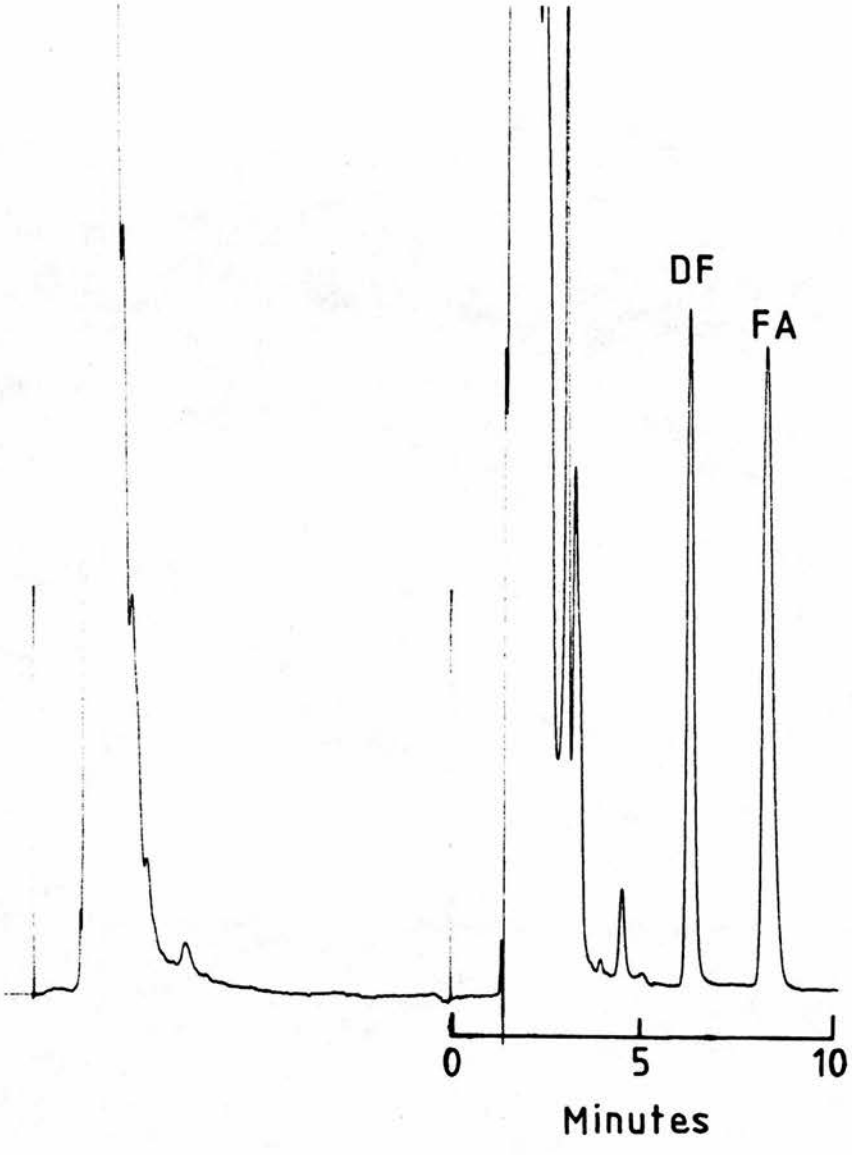

Figure 2.10. A typical chromatogram of urine from a healthy volunteer at time 0 (left) and 3 hours after ingestion of diflunisal (right). DF = diflunisal, FA = flufenamic acid (internal standard).

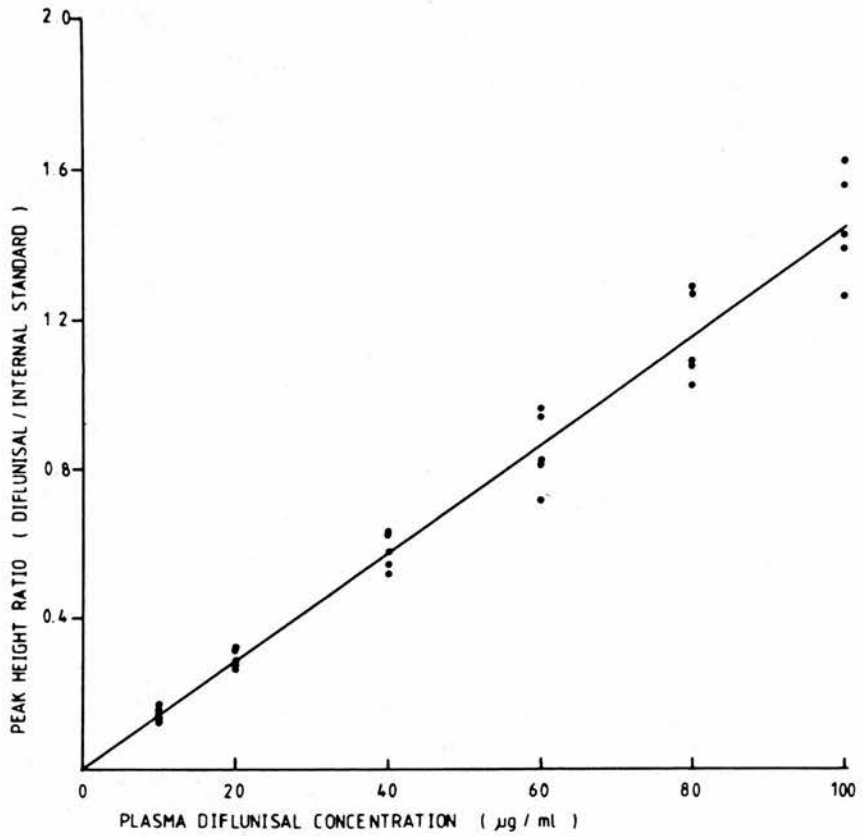

Figure 2.11. Calibration graph for the estimation of diflunisal in plasma, using high performance liquid chromatography with flufenamic acid as internal standard.

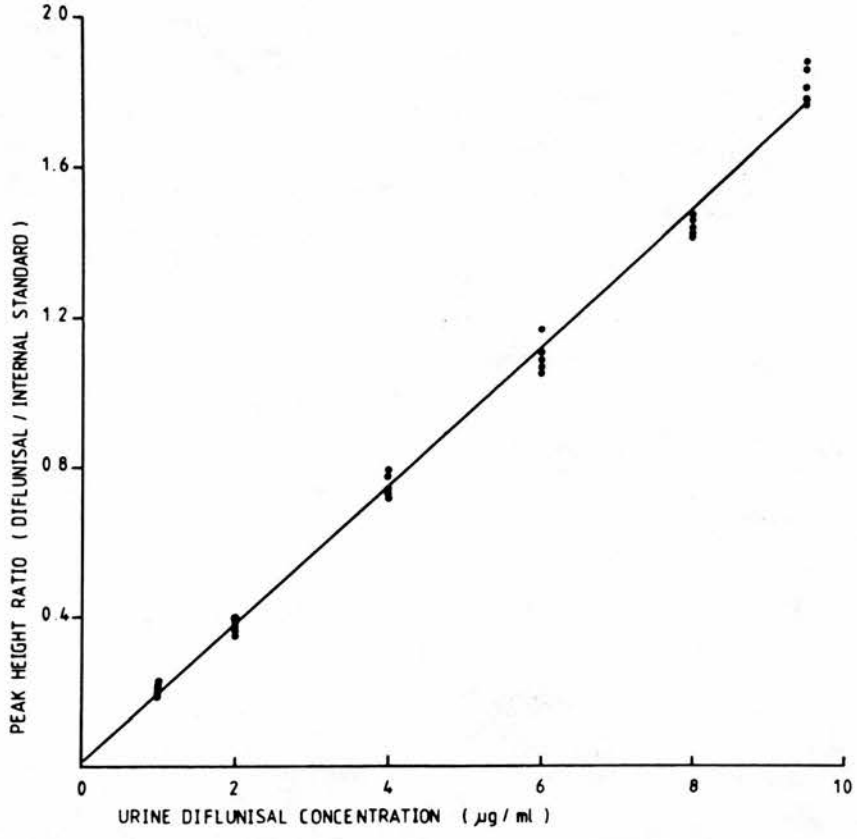

Figure 2.12. Calibration graph for the estimation of diflunisal in urine using high performance liquid chromatography with flufenamic acid as internal standard.

TABLE 2.7. REPLICATE ANALYSES OF DIFLUNISAL IN PLASMA

Drug added (μg/ml)	Drug found (μg/ml) Individual values					Mean	S.D.	Coefficient of variation %
	Run 1	Run 2	Run 3	Run 4	Run 5			
10	9.02	10.66	11.01	10.14	10.33	10.23	0.75	7.33
20	20.45	19.92	20.22	19.70	20.78	20.21	0.43	2.13
40	40.76	39.64	39.37	41.74	40.83	40.50	0.97	2.40
60	59.81	59.37	59.23	58.64	56.08	58.63	1.48	2.52
80	80.76	79.71	78.38	78.49	81.76	79.82	1.46	1.83
100	99.17	100.68	101.78	101.27	100.22	100.62	1.00	1.00

TABLE 2.8. REPLICATE ANALYSES OF DIFLUNISAL IN URINE

Drug added (μg/ml)	Drug found (μg/ml) Individual values					Mean	S.D.	Coefficient of variation %
	Run 1	Run 2	Run 3	Run 4	Run 5			
1	1.09	0.89	1.05	1.11	1.16	1.06	0.10	9.43
2	1.97	1.80	2.07	1.93	1.92	1.94	0.09	4.64
4	4.06	4.22	3.94	4.32	4.00	4.11	0.16	3.90
6	5.87	6.37	5.80	5.56	5.96	5.91	0.30	5.08
8	7.79	8.00	8.01	7.84	7.71	7.87	0.13	1.65
10	10.20	9.69	10.10	10.22	10.22	10.09	0.23	2.28

TABLE 2.9. PLASMA AND URINE CONCENTRATIONS OF DIFLUNISAL IN PATIENTS FOLLOWING OVERDOSAGE MEASURED BY THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

Patient	Source	Number of "Dolobid" tablets	Other drugs taken	Hours after ingestion	Plasma diflunisal concentration (μg/ml)	Hours after ingestion	Urine diflunisal concentration (μg/ml)
RH	Falkirk	50	30 prochlorperazine (5 mg)	3	348	N.A.*	2.2
			30-40 dihydrocodeine	7	251	N.A.	0.9
				25.5	123	N.A.	0
SG	Ward 3, R.I.E.**	30	20 aspirin/codeine tablets	2.75	103	-	-
				25	39	-	-
AD	Manchester	70	-	37	172.5	37	57
ML	Hull	30	2 pints beer	12	131	13-19	82
				18	103	19-25	50
				24	80	23-31	37.5
				30	70	31-43	25.5
				36	44	43-55	30.3
				48	19	55-67	12.4
				60	6	67-79	5.3
				72	1.8	79-81	1.8
				84	trace	81-93	trace
JL	East Kilbride	?	? paracetamol plus d-propoxyphene	?	134	-	-
				?+8	300	-	-

* N.A. = not available. ** R.I.E. Royal Infirmary of Edinburgh.

ingestion of diflunisal. The peak did not change after hydrolysis of the urine with glusulase, whereas thin layer chromatography showed that the glucuronide conjugates of diflunisal disappeared. The interfering peak appeared to be an unknown metabolite of diflunisal.

(c) Comparison of fluorometric and high performance liquid chromatographic methods

The high-performance liquid chromatographic method was compared with the following fluorometric assay which is a modification of the method described by Tocco et al. (1975).

Procedure

To 100 µl of plasma containing 1-30 µg/ml diflunisal in a 15 ml round bottomed glass tube was added 900 µl blank pooled plasma. Appropriate dilutions were made for plasma containing high concentrations. After mixing, 1 ml of 5M hydrochloric acid was added followed by extraction into 5 ml of chloroform. After centrifugation, 2 ml of the chloroform phase was extracted with 3 ml of 0.1 M phosphate buffer pH 8.0. The fluorescence of the aqueous phase was measured in an Aminco-Bowman Spectrophotofluorometer set at 260 nm (activation) and 425 nm (emission).

Concentrations were determined by reference to a previously constructed calibration graph of transmission plotted against plasma diflunisal concentrations obtained from 5 different sets of plasma standards run through the procedure.

The method could not be used for the assay of unchanged diflunisal in the urine, because of interference by glucuronide conjugates. However, /

However, both unchanged and conjugated diflunisal can be measured after hydrolysis with perchloric acid (Tocco et al., 1975).

Results

The plot of per cent transmission minus the blank value versus the plasma concentrations of diflunisal passed through the origin and was linear up to 30 μg/ml. The limit of sensitivity was 0.5 μg/ml.

Plasma concentrations in 3 healthy volunteers after oral administration of 750 mg diflunisal and 3 patients with diflunisal overdosage were measured by both methods. (Figure 2.13). There was excellent agreement.

Conclusion

Both the high performance liquid chromatographic and fluorometric methods are suitable for the estimation of diflunisal in plasma. However, the high performance liquid chromatographic assay has the advantages of simplicity, specificity and may also be used to measure diflunisal in urine.

(d) Summary

Simple, rapid, specific and sensitive high performance chromatographic methods were developed for the estimation of diflunisal in plasma and urine. The mobile phase consisted of 0.08 M potassium nitrate in 2% acetic acid, isopropanol and ethyl acetate (55:25:20). Flufenamic acid was used as the internal standard and acetone used for the precipitation of plasma proteins. After centrifugation, the clear supernatant was injected directly into the chromatograph.

Urine /

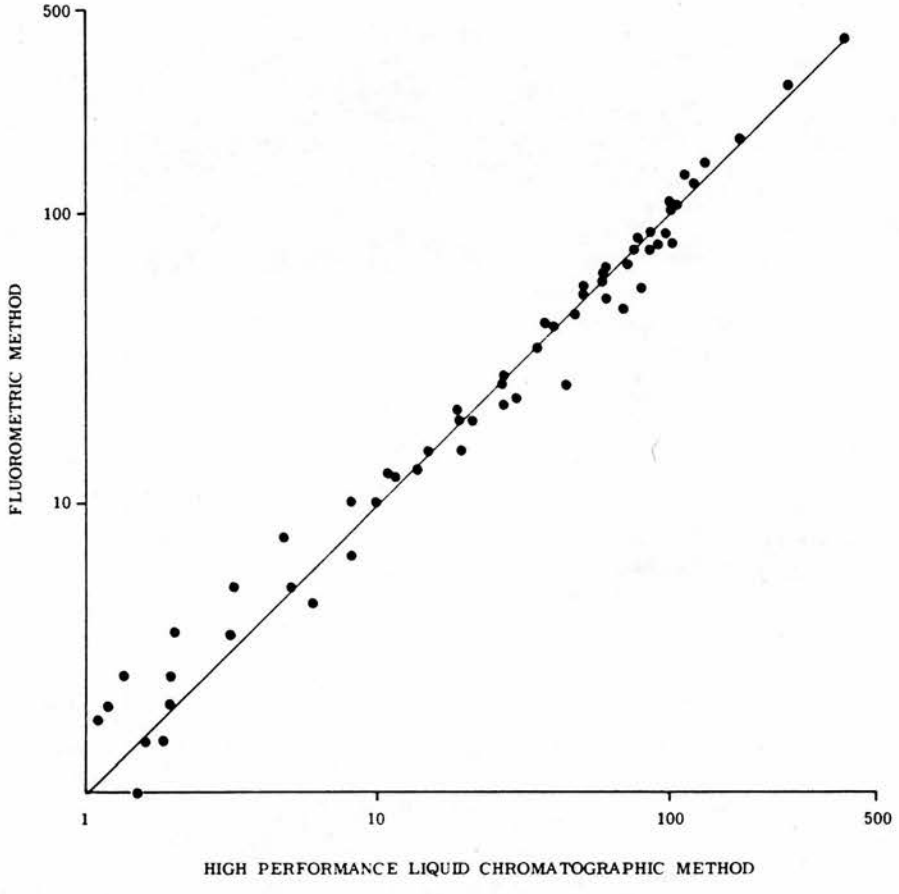

Figure 2.13. Comparison of high performance liquid chromatographic and fluorometric methods for the estimation of diflunisal in 59 plasma samples. The individual measurements (μg/ml) were plotted on logarithmic graph paper with the line of identity (45°).

The urine assay simply involved adding the internal standard, mixing and injecting directly into the chromatograph.

The standard calibration graphs for plasma and urine were linear and passed through the origin. Good separation and reproducibility were obtained. The limits of sensitivity for the plasma and urine assays were 1.0 and 0.5 μg/ml respectively.

A modified fluorometric assay was also used for the estimation of plasma diflunisal and compared with the high performance liquid chromatographic method. The results were in excellent agreement. The high performance liquid chromatographic method has the advantages of simplicity, specificity and may also be used to measure diflunisal in urine.

SECTION II

Chapter 3.

PLASMA PROTEIN BINDING OF SALICYLIC ACID

(a) Review of the literature

A thorough understanding of the nature and magnitude of drug-protein binding is clearly fundamental to an accurate prediction of therapeutic and toxic effects (Bridges and Wilson, 1976). Several methods have been employed to measure plasma protein binding including equilibrium dialysis, ultracentrifugation, ultrafiltration and gel filtration. None of these methods has a clear superiority over the others (Rowland, 1980), although equilibrium dialysis gave the best measure of binding capacity in one study (McArthur and Smith, 1969).

Smith et al. (1946) studied salicylate protein binding by ultrafiltration and concluded that most of the salicylate in human plasma is bound to the non-diffusible component, presumably plasma proteins. In one study it was concluded that salicylate binds only to plasma albumin and variation in the binding capacity of different sera for salicylate is entirely dependent on changes in the albumin concentration (Reynolds and Cluff, 1960). In another investigation it was concluded that salicylate is predominately bound to albumin in normal subjects but in patients with abnormal plasma protein there is less binding of salicylate to albumin and more binding to pre-albumin (Batterman, Mouratoff, Karler and Tauber, 1962).

The binding of salicylic acid and related compounds to purified proteins was studied by Davison and Smith (1961). They concluded that /

that the carboxylic acid function was the primary site of binding. With most of the compounds there were two types of binding sites, one of relatively high affinity but smaller in number, the second of low affinity but in greater number. Wosilait (1976) showed by theoretical analyses that at low salicylic acid concentrations (up to 14.5 μg/ml) the high affinity sites (site 1) would bind most of the drug, but as the concentration increased these sites approached saturation and the low affinity (site 2) would bind increasing amounts of salicylate. He also concluded that if the protein concentration was reduced, the total amount of drug bound would decrease. Yacobi and Levy (1977) also found a statistically significant negative correlation between the concentration of albumin and unbound salicylic acid. Similar results were obtained by Boobis and Chignell (1979). Decreased salicylate binding in cutaneous hepatic porphyria is also due to reduced albumin concentration (Steele, Boobis, Moore, Goldberg, Brodie and Sumner, 1978).

The binding of salicylate to plasma proteins was studied in different species (Sturman and Smith, 1967). Binding was low in the baboon, dog, rat, mouse, turkey and toad, but much higher (45-95%) in the rhesus monkey, rabbit and guinea-pig. Salicylic and acetylsalicylic acids bind more strongly to bovine serum albumin than to human serum albumin (Aarons, Clifton, Fleming and Rowland, 1980).

The binding of acetylsalicylic and salicylic acids was measured using equilibrium dialysis and gel filtration (Amir Ali and Routh, 1969). In each case binding equilibrium was reached in 4-8 hours for salicylic acid, but a progressive increase in binding of acetylsalicylic acid was found up to 53 hours. In a similar study it was shown that salicylic acid and acetylsalicylic acid binding equilibria were reached within 4-12 and 120-150 hours respectively (Kramer and Routh, 1973).

The /

The unbound fraction of salicylic acid increases with increasing total drug concentration both in vitro and in vivo (Smith et al., 1946; Spector, Torkin and Lorenzo, 1972; Esktrand, Alvan and Borga, 1979; Rowland, 1980), but the concentration ranges were limited and no data are available at total plasma salicylate concentrations above 512 µg/ml.

Indomethacin, ibuprofen, phenylbutazone and warfarin in concentrations of 100 µg/ml displace a significant amount of salicylic acid (10-500 µg/ml) bound to human serum albumin and thus may enhance its therapeutic and toxic effects (Muirden, Deutschman and Phillips, 1974). In vitro addition of heparin to plasma had no quantitively significant effect on the protein binding of salicylate and an in vivo effect was reversible by treating the plasma with activated charcoal, a procedure known to remove fatty acids from albumin (Wiegand and Levy, 1979).

A study in dogs indicated that serum unbound salicylate concentrations closely reflect those in cerebro-spinal fluid. As serum pH declines, cerebro-spinal fluid salicylate concentrations increase and free plasma salicylate decreases as a result of decreasing ionisation of salicylate (Reed and Palmisano, 1975).

In normal subjects, the total body clearance of salicylic acid changed relatively little over the therapeutic range because the increasing fraction unbound compensated for the decreasing clearance of unbound drug (Furst, Tozer and Melmon, 1979).

Salicylate binding to synovial fluid proteins was lower than to plasma proteins over the therapeutic range, particularly at lower drug concentrations which could mainly be due to decreasing affinity of binding to site 1 (Trnavska and Trnavsky, 1980). The protein binding /

binding of salicylic acid is the same in heparinized plasma and serum from the same healthy adult subjects. (Wiegand, Hintze, Slattery and Levy, 1980).

In conclusion, albumin is not the only plasma protein to which salicylate binds, but there are no data available to show its contribution. Salicylate binding to albumin and other plasma proteins is concentration dependent and again the data are limited especially at high concentrations (Smith et al., 1946).

(b) Estimation of salicylic acid binding to plasma proteins

(i) Preparation of fresh plasma

Since the amount of heparin in the lithium heparin tube is very small (12.5 U/ml) and there is no in vitro interaction between heparin and salicylate (Wiegand and Levy, 1979), 120 ml of blood was taken into these tubes from 3 healthy male volunteers aged 28-46 years who had not taken any drugs for at least 2 weeks. The plasma was separated and immediately used for the in vitro protein binding studies.

(ii) In vitro binding

Solutions containing 250, 500, 1000, 2000, 3000 and 4000 µg/ml of salicylic acid in 0.01 M saline phosphate buffer pH 7.4 were made. To each 2 ml of fresh plasma in a conical glass tube was added 0.5 ml of salicylic acid solution to give final concentrations of 50, 100, 200, 400, 600 and 800 µg/ml. After mixing, the samples were incubated at room temperature for at least ½ hour, then 100 µl from each was removed for the estimation of total drug concentration by high performance /

performance liquid chromatography (Chapter 1, Section II). The non-protein bound drug was separated by ultrafiltration using Amicon Centriflo membrane cones (Type CF-25) as follows:

1. The cones were soaked in distilled water before use for at least one hour.
2. Each membrane cone was locked into place in the support.
3. The support was pushed into a short boiling tube, seating the flange on the tube. The tube was then centrifuged at 420 X g for 10 minutes to remove excess water.
4. The boiling tubes were replaced by clean dry tubes and 2.4 ml of plasma was placed in the cones. The cones were then spun at 700 X g for 30 minutes. The volume of ultrafiltrate was 0.5 to 1.5 ml.
5. Each filtrate was tested with Albustix^{R} and if protein was present (> 0.3 g/L), the cone and sample were discarded.
6. The ultrafiltrate was then assayed by high performance liquid chromatography in the usual manner to determine the plasma free (unbound) salicylic acid concentration (D_f).
7. The fraction bound (B) was then calculated from the unbound and total (D_t) salicylic acid concentrations :

$$B = \frac{D_t - D_f}{D_t}$$

8. After use the filter cones were rinsed with distilled water and soaked in 5% (W/V) sodium chloride solution for an hour followed by several rinses with distilled water. The cones were stored in 0.1% (W/V) sodium azide.

(iii) /

(iii) In vivo binding

Ten ml blood samples were taken from patients with aspirin overdosage (Section IV) into lithium heparin tubes. The plasma was separated immediately, 100 µl was removed for the estimation of total salicylic acid by high performance liquid chromatography and 2.4 ml was subjected to ultrafiltration as described above before analysis.

(c) Salicylic acid binding to albumin

Human albumin (20 g/dl) prepared by the Scottish National Blood Transfusion Service was diluted with 0.01 M saline phosphate buffer pH 7.4 to give concentrations of 4 and 5 g/100 ml.

The procedure for the determination of the binding of salicylic acid to human albumin was the same as described above (ii).

(d) Salicylic acid binding to the membrane cone

Procedure

1. Stock solutions of salicylic acid in 0.01 M phosphate buffer pH 7.4 were prepared (50, 100, 200, 400, 600 and 800 µg/ml).
2. The membrane cones (6 for each run) were prepared as described above.
3. 2.4 ml of each of the above stock solutions was added to the cones.
4. The cones were spun at 700 X g for 30 minutes.
5. The ultrafiltrates and the stock solutions were assayed for salicylic acid by the high performance liquid chromatographic method. The values represented the free (D_f) and total (D_t) drug concentrations respectively.

6. /

6. The fraction bound to the membrane cone (β) was calculate as :

$$\beta = \frac{D_t - D_f}{D_t}$$

7. The procedure was repeated twice with new and used cones.
8. The mean values for the fraction bound to the membrane cones (β) was subtracted to give a corrected value for salicylic acid binding to plasma proteins and albumin.

Results

The binding of salicylic acid to new membrane cones was lower than to the used cones (Table 2.10). The mean binding of salicylic acid to new and used cones not more than 3 times increased from 6.5% at 50 µg/ml to 16% at 800 µg/ml. The membrane cones used more than 3 times for salicylic acid binding to plasma proteins or albumin had 25-30% binding at 800 µg/ml.

The greater salicylic acid binding to used membrane cones could be due to the presence of protein residues, which were not removed by washing.

Effects of salicylic acid concentration on protein binding

The effects of salicylic acid concentration on the binding in vitro and in patients with aspirin overdosage are shown in Table 2.11 and 2.12. The results of *in vitro* study showed that as the total salicylic acid concentrations increased from 50 µg/ml to 800 µg/ml, the bound fraction decreased from 86% to 20%. The protein binding of salicylic acid in plasma obtained from 7 patients with aspirin overdosage (in vivo study) decreased similarly from 81 to 23% as /

TABLE 2.10.

PERCENTAGE OF SALICYLIC ACID BOUND

TO THE MEMBRANE CONES

Salicylic acid concentrations (µg/ml)	New cones	Used cones
50	5.1	8.0
100	6.8	8.9
200	8.1	9.9
400	9.4	12.2
600	9.6	14.1
800	12.4	19.3

Two new and 2 used cones are used at each concentration and results expressed as means

TABLE 2.11.

COMPARISON OF SALICYLIC ACID BINDING TO HUMAN ALBUMIN AND TOTAL PLASMA PROTEIN IN VITRO

Salicylic acid concentrations (μg/ml)	% unbound		Ratio unbound to albumin/total plasma protein
	Human albumin	Total plasma protein	
50	52.2 ± 2.7	13.9 ± 1.3	3.7
100	59.1 ± 3.3	17.2 ± 1.3	3.4
200	65.3 + 4.5	27.2 ± 1.1	2.4
400	74.8 ± 4.3	46.8 ± 3.6	1.6
600	81.5 ± 5.2	61.9 ± 5.7	1.3
800	90.2 ± 5.1	79.6 ± 3.4	1.1

Values are given as mean ± standard deviations

TABLE 2.12.

THE BINDING OF SALICYLIC ACID TO PLASMA PROTEINS IN RELATION TO TOTAL SALICYLIC ACID CONCENTRATIONS

Patient and sample No.	Salicylic acid concentration (µg/ml) Total	unbound	% unbound
52-9	106	22	21
52-8	112	23	21
50-8	122	24	19
53-6	192	42	22
58-10	194	77	40
55-8	223	61	27
58-9	225	100	45
55-7	237	64	27
53-5	244	55	23
59-5	313	125	40
59-4	328	140	43
59-6	336	129	39
59-3	359	171	48
58-5	369	211	57
58-4	373	223	60
54-4	400	249	62
58-3	423	286	68
58-2	457	329	72
54-3	473	330	70
54-1	571	441	77
54-2	587	439	75

as the total plasma salicylic acid concentrations increased from 120 to 571 μg/ml respectively.

The unbound fraction of salicylic acid in plasma increased linearly with the plasma concentration both *in vitro* and *in vivo* (Figs. 2.14 and 2.15). The binding *in vitro* and *in vivo* was similar and the regression lines (Figs. 2.14 and 2.15) were compared by covariance analysis (Snedecor and Cochran, 1976). There was no significant difference (F variance ratio = 0.0271, $p > 0.05$).

(e) Differences in binding of salicylic acid to total plasma protein and human albumin

The mean percentages of salicylic acid unbound to human albumin and to total plasma proteins at different drug concentrations are given in Table 2.11. As shown by the ratios of drug unbound to albumin:unbound to total plasma proteins, binding to the latter increased disproportionately at lower salicylate concentrations. Thus there was less binding to albumin than to other proteins at low concentrations.

The fraction bound to albumin decreased from 48% at a salicylic acid concentration of 50 μg/ml to 10% at 800 μg/ml, whereas the binding to total plasma protein at these concentrations was 86% and 20% respectively. The relationship between the fraction bound to albumin and the salicylic acid concentration was not linear (Fig. 2.16). The binding to other proteins was calculated from the difference between the binding to albumin and total plasma protein.

Albumin /

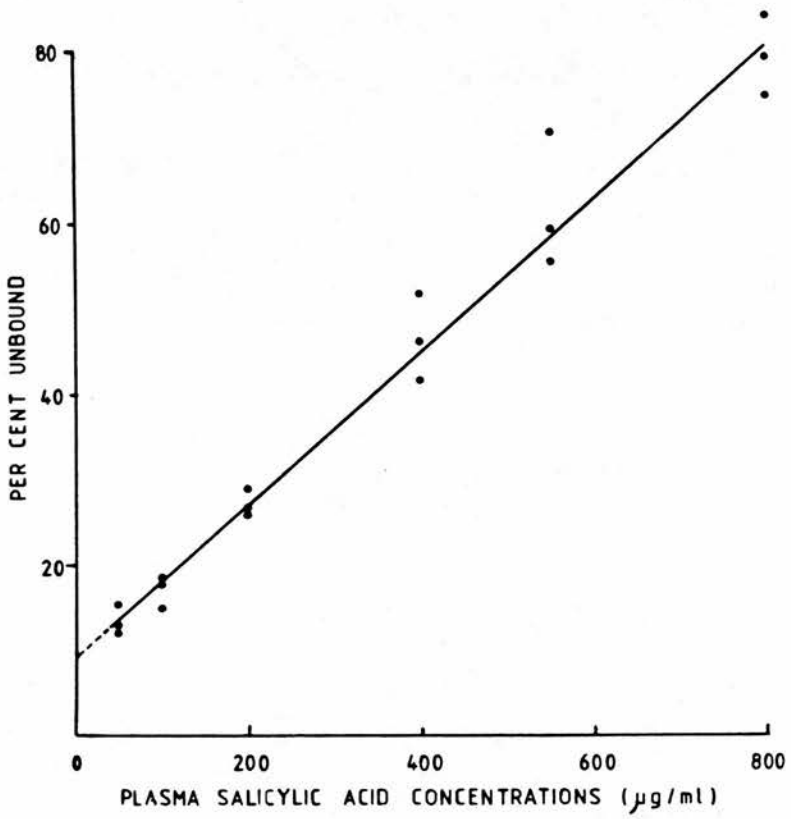

Figure 2.14. Concentration-dependent binding of salicylic acid to plasma proteins (in vitro).

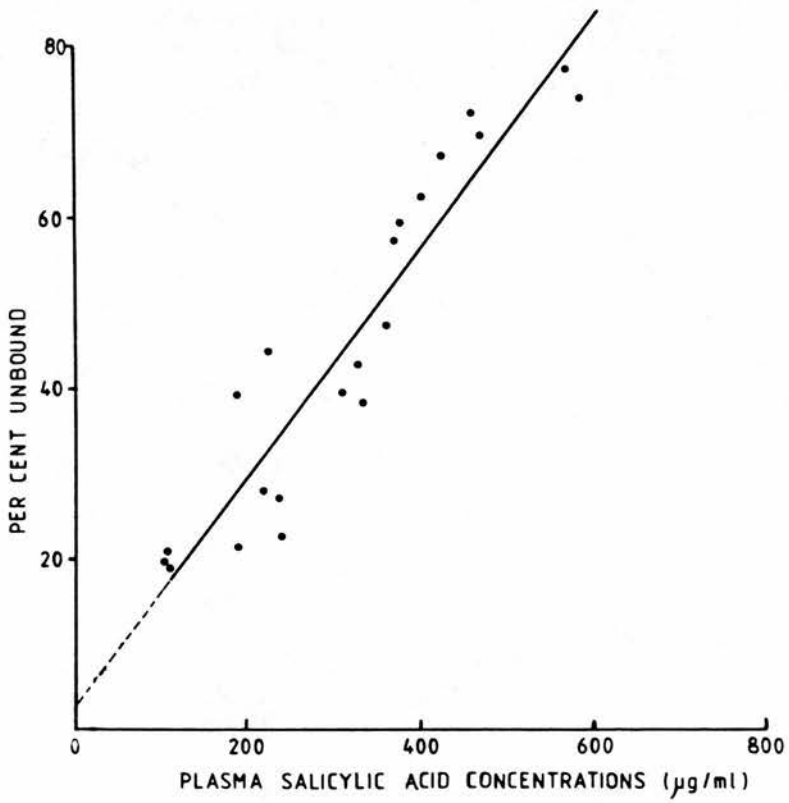

Figure 2.15. Concentration-dependent binding of salicylic acid to plasma proteins. The samples were obtained from the patients with aspirin poisoning.

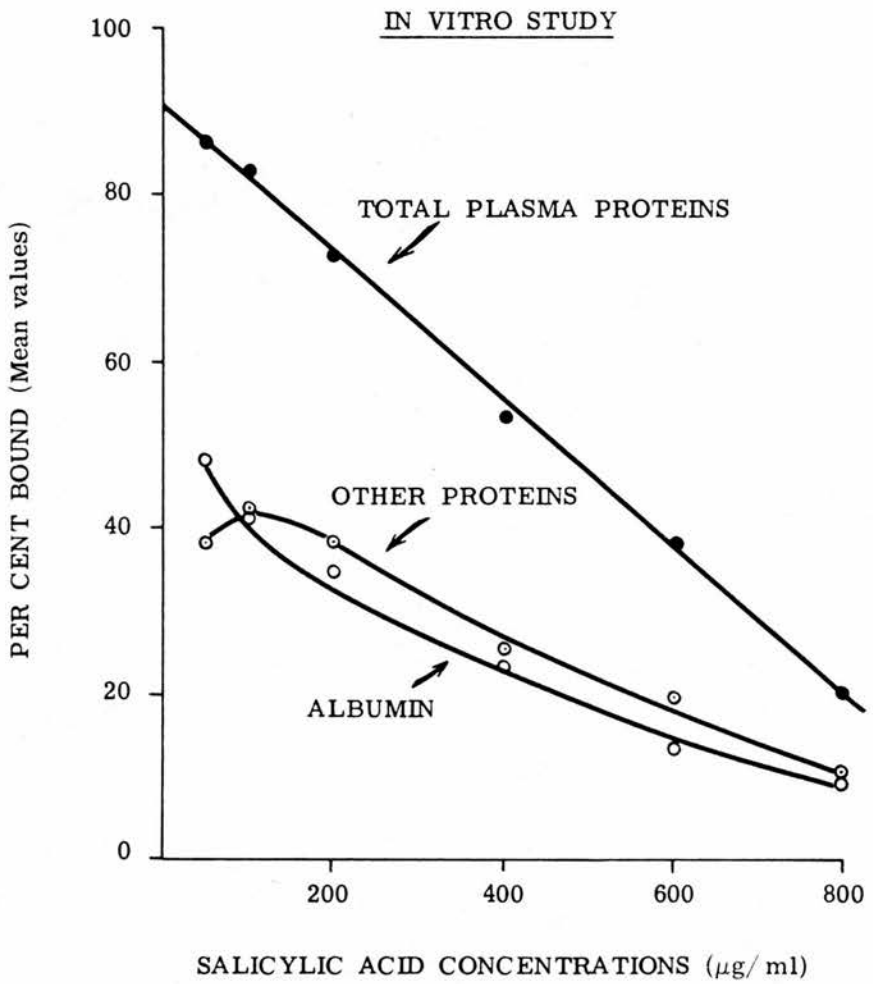

Figure 2.16. Binding of salicylic acid to human albumin and total plasma proteins at different salicylic acid concentrations. The binding to other proteins was calculated from the differences between binding to total plasma protein and human albumin.

Albumin binding accounted for 50% and 55% of the total binding to plasma proteins at concentrations of 800 μg/ml and 50 μg/ml of salicylic acid respectively.

(f) Summary and conclusions

The binding of salicylic acid to plasma proteins in 3 healthy volunteers and 7 patients with aspirin overdosage (in vivo) was studied and compared with in vitro binding to human albumin. The fraction unbound to total plasma proteins increased as the plasma salicylic acid concentrations increased and was similar both in vitro and in vivo. The binding of salicylic acid to albumin accounted for about half of the binding to plasma proteins.

SECTION III

THE EFFECTS OF CHANGES IN URINE pH AND FLOW RATE ON THE PHARMACOKINETICS AND ELIMINATION OF A THERAPEUTIC DOSE OF ACETYLSALICYLIC ACID AND A COMPARISON WITH DIFLUNISAL

SECTION III

Chapter 1.

DISPOSITION OF ACETYLSALICYLIC ACID IN HEALTHY VOLUNTEERS AND THE EFFECTS OF CHANGES IN URINE pH AND FLOW RATE

(a) Introduction

Until recently there have been no simple specific and sensitive methods for monitoring acetylsalicylic acid and its metabolites in plasma and urine. Since high performance liquid chromatographic methods have been developed to measure the acetylsalicylic acid and its metabolites, no comprehensive work on the pharmacokinetics of the drug in healthy volunteers has been reported.

Studies on the effects of changes in urine pH and flow rate on salicylate elimination started last century, but different methods were used for alkalinisation and the results were controversial. Smith et al. (1946) in a comprehensive study, confirmed that alkalinisation of urine increases salicylic acid elimination. Since then, this aspect was studied by other workers (see pages 26-28), but again the methods they employed to estimate the drug and its metabolites were not specific and the word "salicylate" was used in very general terms, thus causing confusion.

This study was undertaken to investigate the disposition and elimination of acetylsalicylic acid in healthy volunteers after a single oral dose of aspirin under different conditions of urine pH and flow rate.

(b) Methods /

(b) Methods

Six healthy ambulant male volunteers aged 24-37 years weighing 63-80 kg were studied with informed consent. They had not taken any drugs for at least two weeks before the study. Each took 20 mg/kg aspirin dissolved in 200 ml of water following an overnight fast. Food, fluids and tobacco were withheld for 2 hours after. Venous blood (5 ml) was taken at 0, ¼, ½, ¾, 1, 2, 3, 5, 8 and 24 hours in lithium heparin tubes containing 50 µl of a 20% (w/v) solution of potassium fluoride. The plasma was separated immediately and placed in glass tubes containing 50 µl of 20% potassium fluoride with 50 µl of glacial acetic acid and stored at -20°C until analysis. Urine was collected at time 0 (blank) then 2 hourly for 12 hours and from 12 to 24 hours. Approximately 20 ml was kept for immediate measurement of pH and the remainder placed in one or two litre plastic bottles containing 250 µl or 500 µl of 20% potassium fluoride and 250 µl or 500 µl of glacial acetic acid respectively. The pH was measured with a pH meter (Radiometer Model pH M62). The total volume of each sample, including the urine kept for pH measurement was measured and 10-15 ml of the sample with preservative was stored at -20°C until analysis. Normal fluid intake, diet and activity were allowed, but alcohol and other drugs were not taken during the study.

The experiment was repeated in the same volunteers not less than 2 weeks later with oral administration of sodium bicarbonate capsules (3 g 4 times daily) the day before and during the study. The fifth dose of sodium bicarbonate (the first dose on the second day) was given one hour before the aspirin. Fluid intake

was /

was increased to give a urine output of about 4 litres daily.

Acetylsalicylic acid and its metabolites in plasma and urine were estimated by the high performance liquid chromatographic method (Chapter 1, Section II). The plasma elimination half-life, total body clearance, volume of distribution, renal clearance and urinary recovery of salicylic acid and other metabolites (where indicated) were calculated for the control study and for the alkaline diuresis study. The plasma half-life was calculated from the slope of the linear elimination phase by the method of least squares and the area under the plasma-concentration-time curve (AUC) by the trapezoidal rule. The total body clearance was calculated by dividing the dose by AUC. The latter was calculated by adding the AUC_{o-t} and the AUC from the time (t) of last plasma concentration (Cp) to infinity (∞) using the following equation :

$$AUC_{t-\infty} = \frac{C_p \times t_{\frac{1}{2}}}{0.693}$$

where $t_{\frac{1}{2}}$ is the plasma elimination half-life of the drug. The apparent volume of distribution (Vd) was estimated from the following equation :

$$Vd = \frac{TBC \times t_{\frac{1}{2}}}{0.693}$$

where TBC is the total body clearance.

The renal clearance was calculated by dividing the amount of drug or metabolite excreted in the urine during a collection period by the corresponding AUC. The renal clearance of salicylic and salicyluric acids were calculated for each period of urine collection /

collection and for the whole period of study as well.

Comparisons were performed using the paired two tailed Student t-test, taking $p < 0.05$ as the minimum level of statistical significance.

N.B. Since pH is the reciprocal of the logarithm of the hydrogen ion concentration, means and standard deviations could not be calculated directly from each pH value (a common mistake in the medical literature). The pH values were converted to hydrogen ion concentrations, the means and standard deviations were calculated and then converted back to pH values. The values are given as mean and standard deviations unless otherwise stated.

(c) Results

Acetylsalicylic acid absorption

Acetylsalicylic acid was absorbed rapidly with a mean peak plasma concentration of 15 μg/ml at 30 minutes in the control study. With sodium bicarbonate absorption was faster and the mean maximum plasma acetylsalicylic acid concentration was 17 μg/ml at 15 minutes. Both salicylic and salicyluric acids plasma concentrations at 15 minutes were significantly higher ($p < 0.05$) with sodium bicarbonate than with the controls (Fig. 3.1). The mean peak plasma salicylic acid concentrations were reached within 1 and 3 hours with and without sodium bicarbonate respectively. These findings demonstrate faster absorption of acetylsalicylic acid when it was taken 1 hour after sodium bicarbonate.

Plasma /

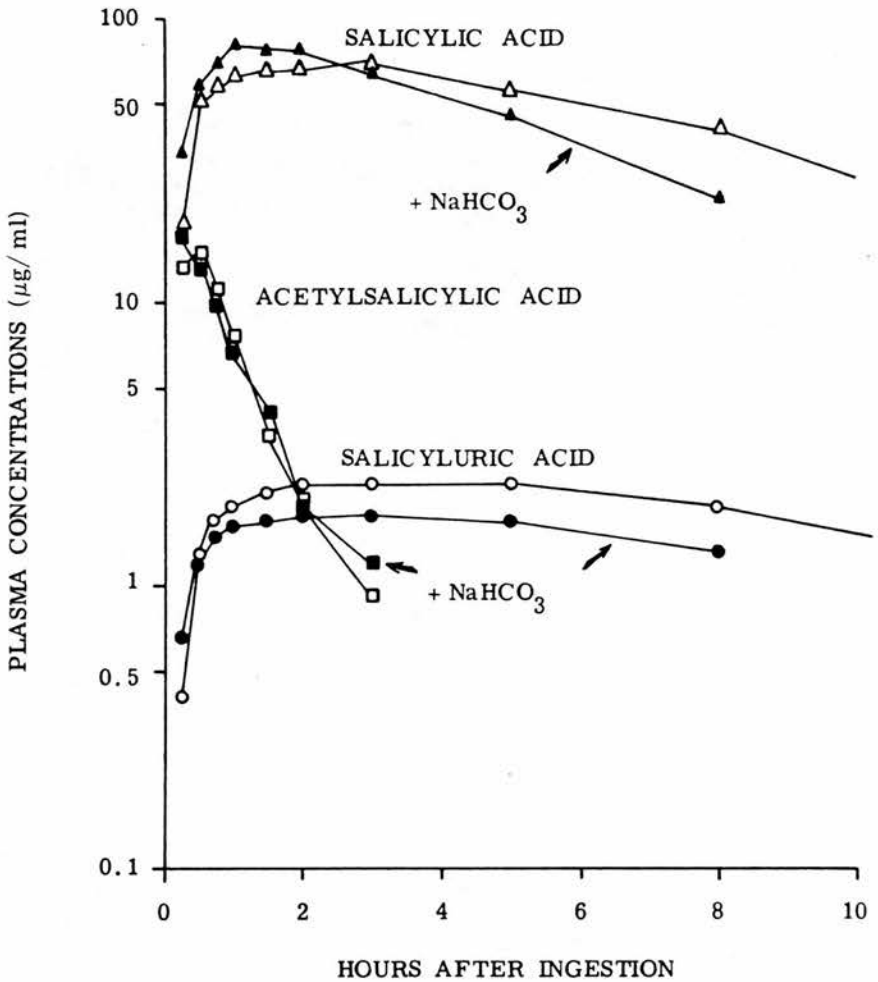

Figure 3.1. Mean plasma concentrations of acetylsalicylic, salicylic and salicyluric acids following a single oral dose of 20 mg/kg of aspirin with and without sodium bicarbonate in 6 healthy subjects.
△, ○, □ = without. ▲, ●, ■ = with sodium bicarbonate.

Plasma concentrations of acetylsalicylic acid and its metabolites

Plasma acetylsalicylic acid concentrations declined very rapidly both with and without sodium bicarbonate and the overall mean half-life was 34 ± 17 minutes (Fig. 3.1.). Acetylsalicylic acid was not detected in plasma (< 0.5 μg/ml) in any subject after 5 hours.

The mean plasma salicylic acid concentrations were higher with sodium bicarbonate than in the control study up to 2 hours after ingestion ($p < 0.001$), but decreased more rapidly subsequently as shown in Figure 3.1. However, the mean areas under the plasma salicylic acid concentration-time curves (0 - 8 hr) were only slightly lower with alkaline diuresis than in the control study (414 ± 88 and 434 ± 51 $\mu g. ml^{-1}$ hr respectively). Salicylic acid was not detected (< 2 μg/ml) at 24 hours in 3 subjects without and in all with sodium bicarbonate.

The mean plasma salicyluric acid concentrations at 15 minutes were 0.66 μg/ml and 0.41 μg/ml with and without sodium bicarbonate respectively ($p > 0.10$), but thereafter were always lower ($p < 0.001$) with sodium bicarbonate (Fig. 3.1.).

Plasma salicylic and salicyluric acid glucuronide conjugates were measured in 3 subjects without administration of sodium bicarbonate. Salicylic acid glucuronide conjugate concentrations varied from 0.68 μg/ml to 53 μg/ml in different samples and accounted for 0 - 2.5 times of the unconjugated salicylic acid. Salicyluric acid glucuronide conjugate concentrations varied from 0.03 to 2.08 μg/ml and accounted for 0 - 1.2 times of the unconjugated salicyluric acid.

Plasma half-life, total body clearance and volume of distribution of salicylic acid

The /

The plasma half-life, total body clearance and volume of distribution of salicylic acid are given in Table 3.1.

The plasma salicylic acid half-life was shorter with the administration of sodium bicarbonate than without in all subjects. The shortest half-life with alkaline diuresis was 2.61 hours whereas the corresponding value in the control study was 4.24 hours. The mean values were 3.56 and 5.66 hours, with and without sodium bicarbonate respectively and this difference was clinically and statistically significant ($p < 0.05$).

The mean total body clearance was significantly increased from 23.2 ml/min in the control to 33.4 ml/min with the administration of sodium bicarbonate ($p < 0.005$). This change in the total body clearance was due entirely to the increased renal clearance of salicylic acid with alkaline diuresis (Table 3.1.).

The apparent volume of distribution of salicylic acid did not change significantly with sodium bicarbonate. The mean values were 147 and 164 ml/kg with and without alkaline diuresis respectively.

Effects of changes in urine pH and flow rate on renal clearance and metabolite excretion

Urine pH and flow rate were significantly higher ($p < 0.001$ and $p < 0.005$ respectively) with the alkaline diuresis than in the control study (7.5 ± 0.1 and 6.3 ± 0.2; 4.20 ± 0.94 and 0.89 ± 0.21 ml/min respectively). The mean urine pH and flow rate for each period /

TABLE 3.1. SALICYLIC ACID PHARMACOKINETICS IN HEALTHY VOLUNTEERS

Subject	Plasma half-life (hr)		Total body clearance (ml/min)		Apparent Volume of distribution (ml/kg)		Renal clearance (ml/min)		Urinary recovery (mg)	
	Control	+$NaHCO_3$	Control	+$NaHCO_3$	Control	+$NaHCO_3$	Control	+$NaHCO_3$	Control	+$NaHCO_3$
M.B.	5.77	5.18	21.2	25.4	132	142	0.56	5.16	31	513
M.L.	8.15	3.00	15.8	31.1	192	139	1.25	13.1	74	367
J.C.	4.24	3.29	22.8	37.7	133	159	2.25	17.6	94	384
H.R.	6.46	3.75	21.7	30.3	180	150	2.12	11.2	87	332
R.A.	4.72	3.52	27.5	38.3	154	160	0.49	11.0	19	324
I.K.	4.61	2.61	30.4	37.3	193	134	6.55	17.1	185	421
Mean	5.66	3.56	23.2	33.4	164	147	2.20	12.5	82	390
S.D.	1.48	0.89	5.13	5.23	28.2	10.8	1.91	3.88	50	59
p value	$0.025 < p < 0.05$		$0.001 < p < 0.005$		$0.30 < p < 0.40$		$p < 0.001$		$p < 0.001$	

period of urine collection with and without sodium bicarbonate are given in Table 3.2.

The renal clearance of acetylsalicylic acid was only measurable in the first 2 hour samples of 5 subjects as shown in Table 3.3. However, the mean values were 7.51 ± 3.96 ml/min and 27.8 ± 12.4 ml/ min before and after sodium bicarbonate respectively and there were significant overall positive correlations between the renal clearance of acetylsalicylic acid and urine pH or flow rate ($r = 0.863$ $p < 0.01$ and $r = 0.917$, $p < 0.01$ respectively).

The renal clearance of salicylic acid was significantly higher ($p < 0.001$) with sodium bicarbonate than without (Table 3.1.) The mean values were 12.5 ± 3.9 and 2.2 ± 1.9 ml/min respectively. As expected, there was an overall highly significant positive correlation between the renal clearance of salicylic acid and urine pH ($r = 0.70$, $p < 0.001$). The correlation was even greater when the renal clearance of salicylic acid was corrected for flow rate ($r = 0.79$) as shown in Figure 3.2. The renal clearance corrected for flow rate is the ratio of concentrations in urine and plasma, which is mathematically independent of urine pH or flow rate. However, there was no significant correlation between the ratio of urine to plasma salicylic acid concentration and urine flow rate ($r = -0.19$).

The renal clearance of salicyluric acid (Table 3.4) was also significantly greater with alkaline diuresis than in the control study (567 ± 84 and 443 ± 117 ml/min respectively, $p < 0.05$). There was a significant positive correlation between the renal clearance of salicyluric acid and urine pH ($r = 0.41$, $p < 0.01$), but not with urine flow rate ($r = 0.23$).

The /

TABLE 3.2. URINE pH AND FLOW RATE IN HEALTHY VOLUNTEERS GIVEN 20 mg/kg OF ASPIRIN WITH AND WITHOUT ALKALINE DIURESIS

Periods of urine collection (hr)	Urine pH		Urine flow rate(ml/min)	
	Control	With $NaHCO_3$	Control	With $NaHCO_3$
0- 2	6.2 ± 0.9	7.7 ± 0.4	0.67 ± 0.36	1.37 ± 0.79
2- 4	6.1 ± 0.5	7.4 ± 0.1	0.70 ± 0.24	6.70 ± 3.82
4- 6	6.5 ± 0.9	7.6 ± 0.4	0.67 ± 0.4	7.43 ± 5.45
6- 8	6.4 ± 0.8	7.6 ± 0.2	0.88 ± 0.45	7.77 ± 5.38
8-10	6.2 ± 0.6	7.5 ± 0.2	1.35 ± 0.63	6.42 ± 4.69
10-12	6.6 ± 0.6	7.4 ± 0.2	1.90 ± 1.57	7.13 ± 2.99
12-24	6.2 ± 0.2	7.4 ± 0.2	1.33 ± 1.32	4.21 ± 3.46
P - value	< 0.001		< 0.005	

TABLE 3.3.

RENAL CLEARANCE OF ACETYLSALICYLIC ACID 0-2 HOURS

Subject	Renal clearance (ml/min)	
	Control	With $NaHCO_3$
M.B.	4.21	13.7
M.L.	6.41	N.M.*
J.C.	11.9	31.5
R.A.	N.M.	23.0
I.K.	N.M.	42.8
Mean	7.51	27.8
S.D.	3.96	12.4

* not measurable

TABLE 3.4.

RENAL CLEARANCE OF SALICYLURIC ACID 0-8 HOURS

Subject	Renal clearance (ml/min)	
	Control	With $NaHCO_3$
M.B.	448	470
M.L.	408	621
J.C.	613	722
H.R.	262	572
R.A.	416	452
I.K.	513	564
Mean	443	567
S.D.	117	84
p - value	< 0.05	

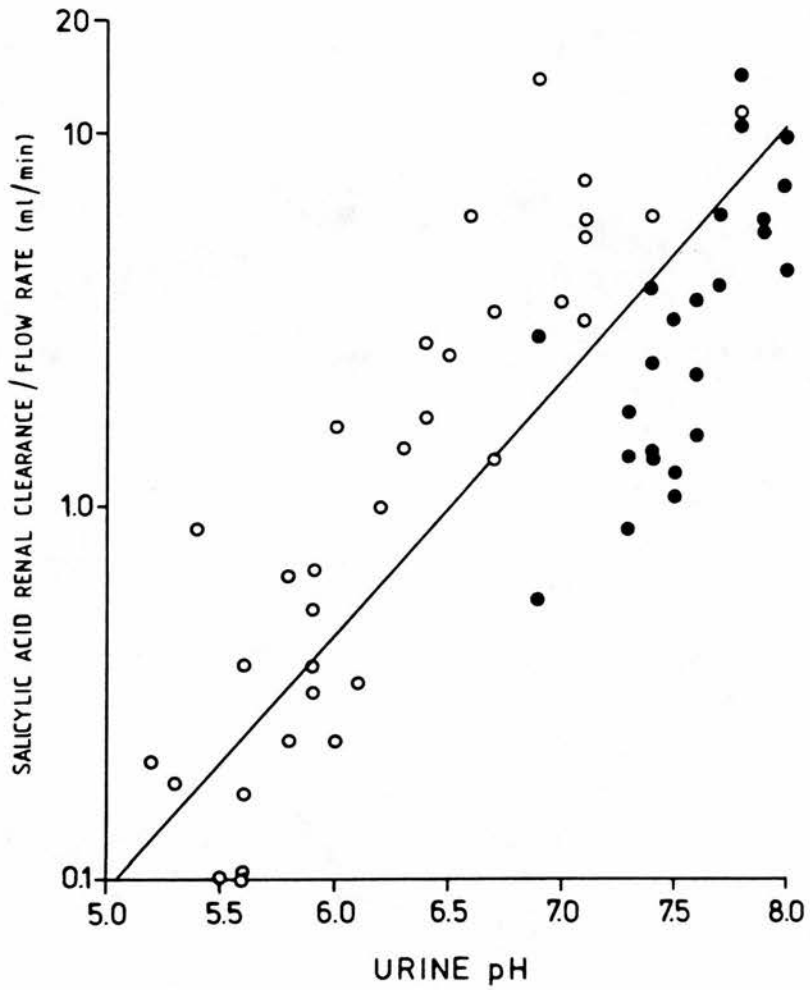

Figure 3.2. Correlation between renal clearance of salicylic acid and urine pH, following a single oral dose of 20 mg/kg of aspirin with (●) and without (○) sodium bicarbonate in 6 healthy subjects.

The urinary recovery of acetylsalicylic acid and its metabolites is shown in Table 3.5. The mean urinary recoveries of acetylsalicylic and salicylic acids were significantly increased from 15 to 26 and 82 to 390 mg respectively with alkaline diuresis ($p < 0.005$ and $p < 0.001$ respectively) but the recovery of salicyluric acid was significantly decreased from 901 to 607 mg ($p < 0.01$). The mean percentage recoveries of acetylsalicylic, salicylic and salicyluric acids (as acetylsalicylic acid) were 1.2, 8.3 and 62 in the control study and 2, 38 and 42 with sodium bicarbonate respectively. However, the total recoveries excluding glucuronide conjugates as the percent of the dose were 82 and 71 with and without sodium bicarbonate respectively ($p < 0.025$).

The ratio of urinary recovery of salicylic to salicyluric acids (as percentage of the dose) increased seven-fold with alkaline diuresis compared with the control study (0.90 and 0.13 respectively). The urinary recovery of gentisic acid was only 1% of the total while glucuronide conjugates accounted for 10%, 21% and 33% of the total in the 3 subjects studied without administration of bicarbonate.

(d) Discussion

Acetylsalicylic acid was absorbed more rapidly when given after sodium bicarbonate and this could be due to better dissolution and more rapid gastric emptying. This is consistent with previous reports (Leonard, 1963; Cooke and Hunt, 1970). Acetylsalicylic acid was hydrolysed very rapidly to salicylic acid and only 1-2% of the

TABLE 3.5. URINARY RECOVERY OF ACETYLSALICYLIC ACID AND METABOLITES IN HEALTHY VOLUNTEERS OVER 24 HOURS

Subject	Condition	Dose (mg)	Acetylsalicylic acid (mg)	% of dose	Salicylic acid (mg)	% of dose	Salicyluric acid (mg)	% of dose	Total % of dose recovered
M.B.	Control	1600	4.74	0.3	30.7	2.5	1104	63.7	66.5
	with $NaHCO_3$	1600	11.33	0.7	513.2	41.8	553.3	31.9	74.4
M.L.	Control	1160	6.57	0.6	74.2	8.3	921.0	73.3	82.2
	with $NaHCO_3$	1160	21.9	1.9	366.5	41.2	581.8	46.3	89.4
J.C.	Control	1260	12.5	1.0	93.6	9.7	946.2	69.3	80.0
	with $NaHCO_3$	1260	24.3	1.9	383.6	39.7	684.9	50.2	91.8
H.R.	Control	1300	21.5	1.6	86.6	8.7	593.0	42.1	52.4
	with $NaHCO_3$	1300	31.2	2.4	331.5	33.2	565.6	40.2	75.8
R.A.	Control	1460	7.27	0.5	19.3	1.7	1119.7	70.8	73.0
	with $NaHCO_3$	1460	22.0	1.5	323.5	28.9	666.6	42.1	72.5
I.K.	Control	1260	37.0	2.9	184.5	19.1	718.8	52.6	74.6
	with $NaHCO_3$	1260	43.7	3.5	420.7	43.5	592.1	43.4	90.4
Mean ± S.D.	Control	20 mg/kg	14.93 ± 12.40	1.15 ± 0.97	81.50 ± 58.80	8.33 ± 6.26	900.50 ± 209.54	62.0 ± 12.21	71.45 ±10.85
	with $NaHCO_3$	20 mg/kg	25.74 ± 10.90	1.98 ± 0.93	389.83 ± 70.08	38.05 ± 5.72	607.40 ± 55.0	42.35 ± 6.20	82.40 ± 9.02
P value		-	$0.001 < p < 0.005$		$p < 0.001$		$0.005 < p < 0.01$		$0.02<p<0.025$

the dose was recovered unchanged in the urine. Although the mean plasma elimination half-life and area under the plasma concentration-time curve of acetylsalicylic acid did not change with sodium bicarbonate, the renal clearance was significantly increased and the renal clearance of acetylsalicylic acid was related to the urine pH and flow rate.

Although the plasma salicylic acid concentrations were greater up to 2.5 hours with sodium bicarbonate, the glycine conjugation of salicylic acid apparently saturated at the same time (2 hr) with and without alkaline diuresis (Fig. 3.1.). The significantly lower area under the plasma salicyluric acid concentration-time curve with sodium bicarbonate could be due to increased renal clearance of salicyluric acid which correlated significantly with urine pH. However, the elimination of salicyluric acid followed zero-order kinetics until the amount of salicylate in the body declined to approximately 360 mg (Levy, 1965b) and the plasma salicylic acid concentration dropped below 50 µg/ml. Since there were highly significant positive correlations between the renal clearance of salicylic acid and urine pH, particularly when corrected for flow rate, the significantly lower plasma half-life and higher total body and renal clearances, and urinary recovery are all dependent on urine pH. The influence of urine pH increased remarkably above 7.0, where it completely dominated the elimination kinetics of salicylic acid. Since the renal clearance is derived from the flow rate ($RC = \frac{CU}{CP}$ flow rate), it is not appropriate to calculate the correlation between the renal clearance and flow rate. The ratio of urine to plasma concentration (CU/CP) is the renal clearance corrected for flow rate which is mathematically (but not biologically) independent of urine flow rate. As expected, there was no /

no correlation between the ratio of urine to plasma concentrations and flow rate.

The ratio of ionized/unionized salicylic acid in the renal tubular fluid increases with increasing pH, thus salicylic acid reabsorption decreases and its urinary excretion is enhanced (Schacter and Manis, 1958; Bedford et al., 1965). Since acetylsalicylic acid has similar pKa to salicylic acid (3.5 and 3.0 respectively) and its renal clearance also correlated well with urine pH, the same mechanism probably applies to acetylsalicylic acid; pH-dependent renal excretion of acetylsalicylic acid has not been described previously.

Although the renal clearance of salicyluric acid was significantly higher with sodium bicarbonate, the ratio of the urinary recovery of salicylic to salicyluric acid increased seven-fold with alkaline diuresis. The significantly lower urinary recovery of salicyluric acid with alkaline diuresis is due to increased total body and renal clearances of salicylic acid. The renal excretion of salicyluric acid involves active renal tubular secretion and conjugation (Levy, Amsel and Elliot, 1969).

(e) Summary

The disposition of acetylsalicylic acid following a single dose of 20 mg/kg aspirin was studied in 6 healthy volunteers with and without alkaline diuresis.

Acetylsalicylic acid was absorbed more rapidly when it was taken one hour after sodium bicarbonate.

The mean plasma half-life of salicylic acid decreased significantly from 5.66 hours in the control to 3.56 hours with alkaline diuresis /

diuresis ($p < 0.05$). The total body clearance of salicylic acid was significantly higher ($p < 0.005$) with sodium bicarbonate than in the control (33.4 and 23.2 ml/min, respectively). This increase was due entirely to an increased renal clearance of salicylic acid following sodium bicarbonate. The volume of salicylic acid distribution was slightly, but not significantly lower with alkaline diuresis than in the control (147 and 164 ml/kg respectively).

The mean renal clearances of acetylsalicylic and salicylic acids increased from 3.75 and 2.2 ml/min in the control study to 18.5 and 12.5 ml/min with sodium bicarbonate, respectively. The mean urinary recoveries of acetylsalicylic and salicylic acids were also increased significantly from 15 and 82 mg in the control to 26 and 390 mg with alkaline diuresis respectively. There were significant positive correlations between the renal clearances of acetylsalicylic and salicylic acids and urine pH and flow rate.

The renal clearance of salicyluric acid was increased from 443 to 567 ml/min with alkaline diuresis, but its urinary recovery decreased from 901 to 607 mg, due to increased salicylic acid elimination. The ratio of the urinary recovery of salicylic to salicyluric acids increased seven-fold with alkaline diuresis.

Alkaline diuresis enhanced the elimination of acetylsalicylic and salicylic acids and the proportion of the dose recovered in the urine as salicyluric acid was correspondingly reduced.

SECTION III

Chapter 2.

THE EFFECTS OF CHANGES IN URINE pH AND FLOW RATE ON DIFLUNISAL ELIMINATION IN HEALTHY VOLUNTEERS

(a) Introduction

Diflunisal (2',4',difluoro-4-hydroxy-3-biphenylcarboxylic acid) is a derivative of salicylic acid (Fig. 1) with similar pharmacology and toxicology (Stone et al., 1977). However, it is longer acting (Tempero, Cirillo and Steelman, 1977) and is claimed to cause less gastrointestinal bleeding (De Schepper and Tjanramaga, 1978) and to have less marked effects on platelet function (Smith Sibinga, 1977) although bleeding from duodenal and gastric ulcers has been reported (Talbot and Rees, 1978; Scott, 1979; Admani and Khaleque, 1979).

Diflunisal is a lipid-soluble, organic acid (pKa 3.3) and as such its renal clearance should be pH-dependent (Milne, 1965). On the other hand, it is very highly bound to plasma proteins, an average of 99.8% at 50 μg/ml (Verbeeck, Boel, Buntinx and De Shepper, 1980). Diflunisal elimination is dose-dependent. The area under the plasma concentration-time curve was 18 times higher following a dose of 500 mg than with 50 mg (Tocco et al., 1975)
The effects of urine pH and flow rate on the renal clearance of diflunisal were studied by conventional clearance techniques in anaesthetised dogs (Baer, Breault and Russo, 1978). The net renal clearance of diflunisal was very low (about 1% of the glomerular filtration rate) and changes in the urine flow or pH had very little effect. About half of a single intravenous dose of diflunisal is excreted in the urine in dogs (50% unchanged and 50% as glucuronide conjugates) /

conjugates), whereas in man, 95% of an oral dose appears in the urine, principally as glucuronide conjugates (Tocco et al., 1975). Verbeeck, Tjandramaga, Mullie, Verbesselt, Verberckmoes and De Schepper (1979) studied the biotransformation of diflunisal and the renal excretion of its glucuronides in renal insufficiency and found that the plasma half-life was increased by a factor of up to ten in patients with renal failure. Levy (1979) pointed out that renal dysfunction would cause a larger fraction of conjugated diflunisal to be excreted in the bile. Only a small fraction of diflunisal (< 5%) is excreted unchanged in the urine in man and it is difficult to see how diflunisal elimination could be enhanced by increasing urine pH and flow rate. Nevertheless, forced alkaline diuresis has been recommended for the treatment of diflunisal overdosage (ABPI Data Sheet Compendium, 1979-1980), although its efficacy is unknown.

The present study was carried out to measure the plasma half-life concentrations, volume of distribution, total body clearance, renal clearance and excretion of diflunisal in healthy volunteers under different conditions of urine pH and flow rate.

(b) Methods

Six healthy ambulant male volunteers aged 20-37 years, weighing 60-83 kg were studied with informed consent. The subjects had not taken any drugs for at least 2 weeks before the study. Each took 750 mg of diflunisal (3 "Dolobid" tablets) with 200 ml of water following an overnight fast after which food, fluids and tobacco were withheld for 2 hours. Venous blood (5 ml) was taken at 0, ½, 1, 2, 3, 4, 8, 12, 24, 36, 48 and 72 hours and urine

was /

was collected at time 0 (blank urine), 2 hourly for 12 hours and then 12 hourly for another 60 hours. Blood samples were placed in lithium heparin tubes and the plasma separated. The volume and pH of each urine sample were measured. The urine and plasma samples were stored at -20°C. Normal fluid intake, diet and activity were allowed, but alcohol and other drugs were not taken during the study.

The experiment was repeated in the same volunteers not less than 2 weeks later with administration of sodium bicarbonate capsules (3 gm 4 times daily) the day before and for 48 hours after the diflunisal. The fifth dose of sodium bicarbonate (the first dose on the second day) was given one hour before the diflunisal. Fluid intake was increased to give a urine output of about 4 litres daily.

Unchanged diflunisal was estimated in plasma and urine by the high performance liquid chromatographic method (Chapter 2, Section II). The plasma elimination half-life, area under the plasma concentration-time curve, total body clearance, volume of distribution and renal clearance of diflunisal were calculated before and during alkaline diuresis as described in Chapter 1 of this Section. Statistical tests were also performed in the same way.

(c) Results

The plasma concentrations of diflunisal were almost identical in both studies (Fig. 3.3.). Peak plasma concentrations were reached within 3-4 hours and the mean values were 112 ± 15 and 118 ± 11 (S.D.) μg/ml with and without sodium bicarbonate respectively. The /

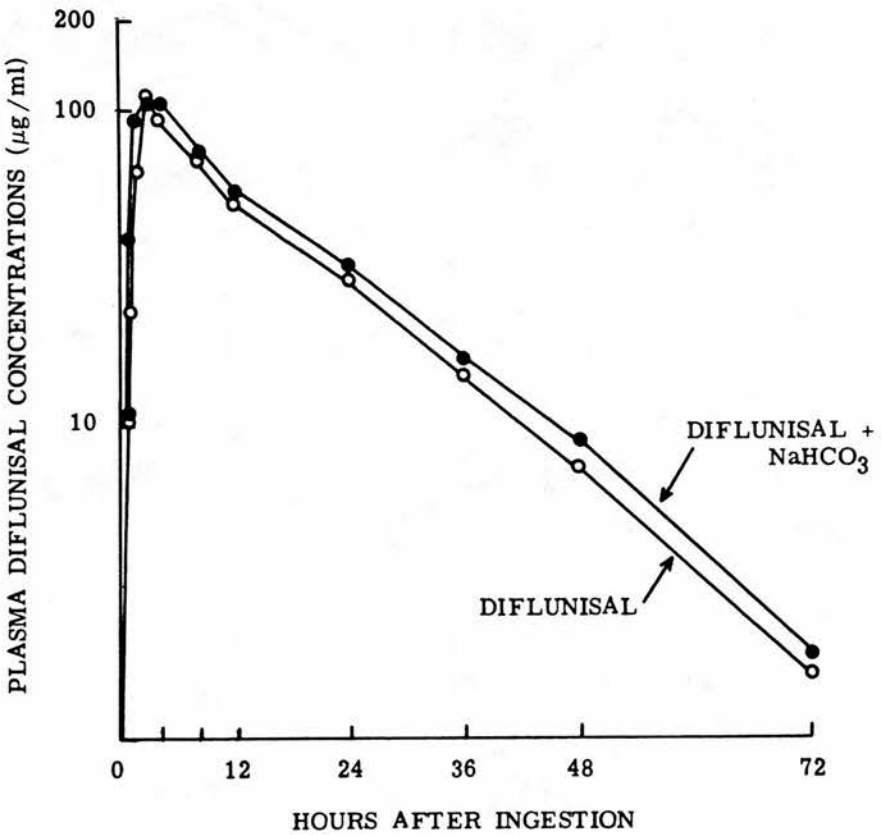

Figure 3.3. Mean plasma concentrations of diflunisal following a single oral dose of 750 mg with and without sodium bicarbonate in 6 healthy subjects.

The effects of alkaline diuresis on the disposition of diflunisal are summarised in Table 3.6. The mean plasma elimination half-life values were virtually identical in both studies (12.9 + 1.8 and 12.5 ± 1.8 hours). If anything, plasma diflunisal concentrations were higher rather than lower during alkaline diuresis. The mean area under the plasma diflunisal concentration-time curve (0-72 hours) was also greater with sodium bicarbonate than in the control study (1954 ± 343 and 1807 ± 207 $\mu g.ml^{-1}$ hr. respectively), but the difference was not statistically significant ($0.20 < p < 0.30$). The total body clearance of diflunisal was lower rather than higher with alkaline diuresis and, surprisingly, its apparent volume of distribution significantly decreased with sodium bicarbonate (Table 3.6.).

Urine pH and flow rate were significantly higher ($p < 0.001$ and $p < 0.005$ respectively) with sodium bicarbonate than the control (pH 7.5 ± 0.4 and 6.3 ± 0.3, flow rate 2.7 ± 0.2 and 0.9 ± 0.2 ml/min respectively).

The mean urine pH and flow rate for each period of urine collection with and without alkaline diuresis are given in Table 3.7.

The 72 hour urinary recovery of diflunisal was more than doubled by alkaline diuresis (Table 3.6), but even so, only 5-7% of the administered dose was excreted unchanged. There was a significant increase in the renal clearance of diflunisal from 0.21 to 0.43 ml/min with alkaline diuresis (Table 3.6), but there was no statistically significant correlation overall between the renal clearance /

TABLE 3.6. EFFECTS OF ALKALINE DIURESIS ON THE DISPOSITION OF DIFLUNISAL

Subject	Plasma elimination half-life (hr)		Total body clearance (ml/min)		Apparent Volume of distribution (ml/kg)		Renal clearance (ml/min)		Urinary recovery (mg)	
	Control	+$NaHCO_3$	Control	+$NaHCO_3$	Control	+$NaHCO_3$	Control	+$NaHCO_3$	Control	+$NaHCO_3$
M.B.	13.7	13.1	8.70	8.06	126	111	0.24	0.75	20.4	68.0
I.K.	10.9	12.5	7.08	5.45	106	94	0.14	0.35	15.2	47.9
H.R.	14.1	12.1	6.20	7.09	116	115	0.11	0.21	13.4	22.2
B.M.	13.6	12.4	6.54	7.20	124	125	0.25	0.46	28.4	47.7
M.K.	10.3	9.7	6.37	5.27	95	74	0.26	0.43	30.9	61.0
R.A.	14.6	15.3	6.41	5.60	110	100	0.23	0.38	26.0	48.8
Mean	12.9	12.5	6.88	6.45	113	103	0.21	0.43	22.4	49.3
S.D.	1.8	1.8	0.94	1.16	12	18	0.06	0.18	7.2	15.6
p value	$0.10 < p < 0.20$		$0.30 < p < 0.40$		$0.025 < p < 0.05$		$0.01 < p < 0.02$		$0.001 < p < 0.005$	

TABLE 3.7. URINE pH AND FLOW RATE IN HEALTHY VOLUNTEERS GIVEN 750 mg DIFLUNISAL WITH AND WITHOUT ALKALINE DIURESIS

Periods of urine collection (hr)	Urine pH		Urine flow rate (ml/min)	
	Control	With Na HCO_3	Control	With Na HCO_3
0- 2	6.4 ± 0.7	8.4 ± 0.2	0.93 ± 0.62	0.97 ± 0.63
2- 4	6.4 ± 0.5	7.4 ± 0.8	0.67 ± 0.39	2.23 ± 1.93
4- 6	6.4 ± 0.5	7.3 ± 0.6	0.78 ± 0.50	4.30 ± 3.50
6- 8	6.3 ± 0.6	7.5 ± 0.3	1.07 ± 0.44	4.39 ± 3.17
8-10	6.1 ± 0.4	7.3 ± 0.4	0.87 ± 0.38	3.28 ± 1.48
10-12	5.9 ± 0.2	7.7 ± 0.5	0.79 ± 0.59	5.93 ± 2.88
12-24	5.8 ± 0.1	7.4 ± 0.4	0.68 ± 0.10	1.97 ± 0.79
24-36	6.4 ± 0.6	7.7 ± 0.2	1.01 ± 0.39	1.96 ± 0.79
36-48	6.7 ± 0.5	7.4 ± 0.3	1.07 ± 0.45	2.07 ± 0.60
48-60	6.2 ± 0.4	7.5 ± 0.2	0.84 ± 0.40	1.95 ± 0.50
60-72	6.5 ± 0.6	6.6 ± 0.3	1.13 ± 0.27	1.10 ± 0.14
P - value	< 0.001		< 0.005	

clearance of diflunisal and urine pH or flow rate ($r = 0.08$ and $r = -0.04$ respectively). Unexpectedly, there was a progressive and highly significant increase in the renal clearance of diflunisal with time (Fig. 3.4) irrespective of urine pH or flow rate ($r = 0.75$, $p < 0.001$ with diflunisal alone and $r = 0.49$, $p < 0.001$ with alkaline diuresis). The overall mean renal clearance of diflunisal increased from 0.22 ml/min over the period of 0-24 hours to 0.73 ml/min from 48-72 hours. There was a correspondingly significant negative correlation between plasma concentrations and renal clearance of diflunisal with and without sodium bicarbonate ($r = -0.50$, $p < 0.001$ and $r = -0.66$, $p < 0.001$ respectively).

(d) Discussion

Although the mean renal clearance and the 72-hour urinary recovery of diflunisal were significantly increased by alkaline diuresis, the total body clearance decreased and only 5-7% of the administered dose was excreted unchanged. There was no reduction in the mean plasma concentrations or half-life. Plasma concentrations were actually higher with alkaline diuresis, possibly due to the increased absorption or decreased volume of distribution. The mean plasma half-life of 12-13 hours is consistent with the previous reports of dose-dependent elimination of diflunisal (Tocco et al., 1975; Tempero, Cirillo and Steelman, 1978). The total body clearance and volume of distribution of diflunisal in the control were also compatible with values cited in the literature (Verbeeck et al., 1979).

We were surprised to find a reduced total body clearance and volume /

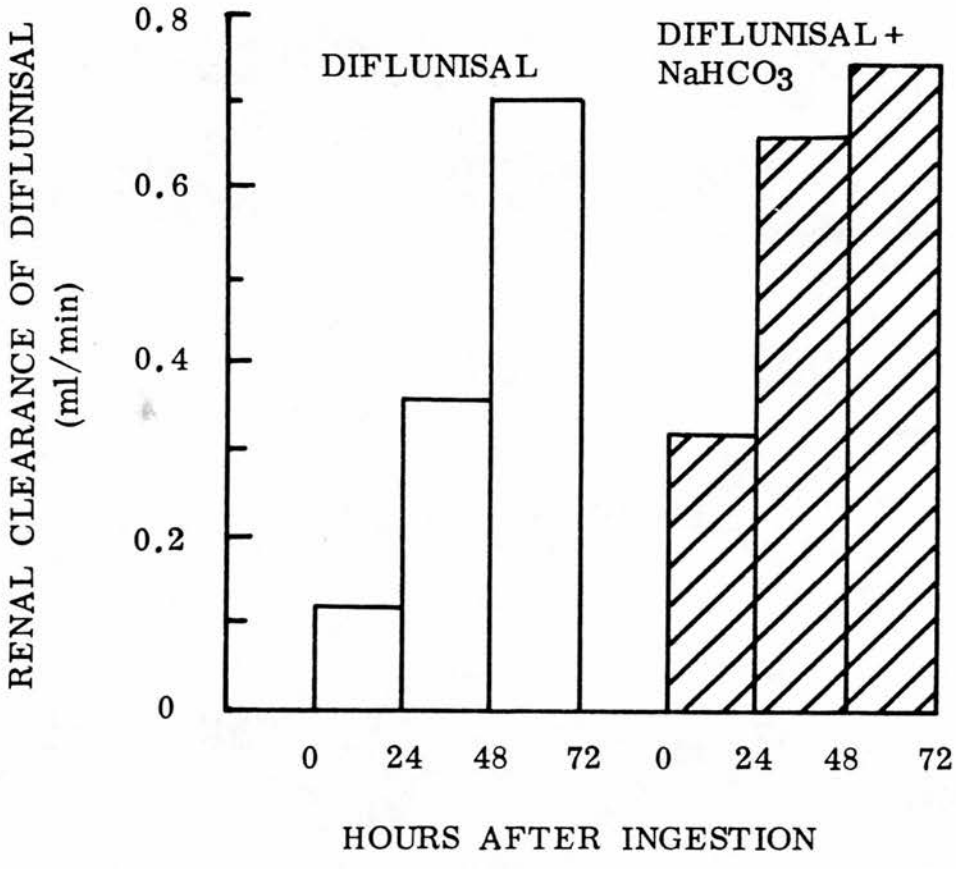

Figure 3.4. The increase in mean renal clearance of diflunisal with time following a single oral dose of 750 mg with and without sodium bicarbonate in 6 healthy subjects.

volume of distribution of diflunisal with alkaline diuresis and no significant overall correlation between the renal clearance of diflunisal and urine pH or flow rate. On the other hand, there was a highly significant relationship between time after administration (and plasma concentration) and renal clearance of diflunisal irrespective of urine pH or flow rate. The explanation for these findings is unknown. Possibilities include induction of renal tubular transport of diflunisal with time, or that the maximum tubular secretory capacity was exceeded at higher plasma concentrations. Similar findings were reported for the renal clearance of salicyl acyl glucuronide (but not salicylic acid) by Schachter and Manis (1958). We have confirmed that there is no correlation between the renal clearance of salicylic acid and its plasma concentrations or time after administration of therapeutic doses of aspirin in healthy volunteers. Interestingly, an opposite relationship has been described between the plasma concentration and renal clearance of disopyramide which was attributed to concentration-dependent plasma protein binding (Cunningham, Shen, Shudo and Azarnoff, 1977). Enhancement of diflunisal elimination by alkaline diuresis would be most unlikely since being very highly bound to plasma proteins, so little is excreted unchanged (Verbeeck and De Schepper, 1980; Tocco et al., 1975).

Forced alkaline diuresis has been recommended for the treatment of diflunisal overdosage. In one report it appeared to have little beneficial effect, although no measurements were made (Upadhyay and Gupta, 1978). According to the results of the present study, forced alkaline diuresis would be a useless treatment for diflunisal poisoning.

(e) Summary /

(e) Summary and conclusions

The effect of alkaline diuresis on the elimination of a single oral dose of 750 mg diflunisal was studied in 6 healthy male volunteers.

The plasma concentrations and half-life of diflunisal were not reduced by alkaline diuresis. The total body clearance and the volume of distribution of the unchanged drug were reduced. The 72-hour urinary recovery of diflunisal was more than doubled, but even so, only 5-7% of the administered dose was excreted unchanged. With alkaline diuresis there was a significant increase in the mean renal clearance of diflunisal from 0.27 to 0.46 ml/min but there was no significant correlation between the renal clearance of diflunisal and urine flow or pH. However, there was a significant increase in the overall mean renal clearance of diflunisal from 0.22 ml/min over the period of 0-24 hours to 0.73 ml/min from 48-72 hours.

Forced alkaline diuresis is of no value in diflunisal poisoning.

SECTION IV

EFFECTS OF CHANGES IN URINE pH AND FLOW RATE ON SALICYLATE DISTRIBUTION AND ELIMINATION FOLLOWING OVERDOSAGE

SECTION IV

Chapter 1.

PHARMACOKINETICS OF ACETYLSALICYLIC ACID IN OVERDOSAGE WITHOUT TREATMENT BY FORCED ALKALINE DIURESIS

(a) Introduction

Although acetylsalicylic acid has been one of the most frequently taken poisons in most countries of the world and hundreds of cases of aspirin overdosage have been reported (e.g. Gross and Greenberg, 1948; Smith, 1966; Kaye, 1972; Locket, 1973; McCleave and Havil, 1974; Mofenson and Greensher, 1975; Bender, 1975; McQueen, 1977; Done, 1978; Proudfoot and Park, 1978), its pharmacokinetics in overdosage have not been investigated in detail. Until recently it was not easy to measure acetylsalicylic acid and its metabolites in plasma and urine (Chapter 1, Section II) and methods employed to monitor salicylate concentrations in patients with aspirin overdosage were not specific.

The present study was undertaken to investigate the disposition and pharmacokinetics of acetylsalicylic acid in 16 control patients with mild aspirin overdosage who received no specific treatment.

(b) Patients and methods

The patients aged 16-65 years and weighing 41-96 kg (5 males and 11 females) were studied on admission to the Regional Poisoning Treatment Centre, The Royal Infirmary, Edinburgh, with peak plasma salicylate concentrations in the range of 300-450 μg/ml. The protocol

was /

was approved by the Physicians' Advisory Ethical Committee. Patients with significant overdosage of other drugs or alcohol and respiratory, cardiac, renal, gastrointestinal and hepatic disease or pregnancy were excluded.

On admission the plasma salicylate concentration was measured by the modified Trinder's method in the ward side room (Chapter 1, Section II) and repeated hourly to determine the peak concentration. The following procedures were performed :

1. The patient was asked to empty his or her bladder. A 20 ml aliquot of urine was placed in a plain tube and the remainder in a one-litre bottle containing 250 µl of 20% (W/V) potassium fluoride and 250 µl of glacial acetic acid. Each urine sample was collected in the same way until the patient was discharged.
2. A No. 19 butterfly cannula with a 2-way tap was inserted in a mid-forearm vein for blood sampling and kept open with a slow-running saline infusion.
3. Blood samples (5 ml) were taken at 0, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36 and 48 hours and placed in lithium heparin tubes containing 50 µl of 20% potassium fluoride.
4. The blood samples were centrifuged immediately. The plasma was placed in a glass tube containing 50 µl of 20% potassium fluoride and 50µl glacial acetic acid and stored at -20°C until analysis.
5. The pH of the 20 ml urine samples was measured using a pH meter and the total volume of each sample measured, 10-15 ml of the sample with preservative was placed in a fresh tube and stored at -20°C until analysis.
6. /

6. Acetylsalicylic acid and metabolite concentrations in plasma and urine were measured by high performance liquid chromatography (Chapter 1, Section II).

The binding of salicylic acid to plasma protein was measured (Chapter 3, Section II) in fresh plasma samples from 2 patients (Nos. 50 and 52). Glucuronide conjugates of salicylic and salicyluric acids were measured in the pooled urine samples of 6 patients.

7. Pharmacokinetic variables were calculated as for the healthy volunteers (Chapter 1, Section III) except for the following :

1. The total body clearance of salicylic acid could not be derived from the dose absorbed and instead was calculated from the following equation :

$$TBC = \frac{RC}{fe}$$

TBC is the total body clearance of salicylic acid, RC the renal clearance and fe the fraction excreted in the urine as salicylic acid. The urine collection periods were very short (average 3 hours) and it was assumed that the change in the excretion rate in each period was linear.

2. The independent two-tailed student t-test, with $p < 0.05$ as the minimum level of statistical significance was used for comparisons.

N.B. Since the periods of urine collection were not the same, the urine flow rate and renal clearance were also calculated 0 to 16 hours after admission in each patient. The mean urine pH in each patient was calculated from the individual samples after conversion to hydrogen ion concentration.

(c) /

(c) Results

Plasma concentrations and elimination of acetylsalicylic acid and its metabolites

Acetylsalicylic acid was detected in the plasma of only 4 patients (Fig. 4.1). The plasma concentrations on admission varied from 3.3 μg/ml at 10 hours to 28 μg/ml at 3 hours after ingestion. Plasma salicylic acid concentrations in these 4 cases increased during the first few hours (up to 16 hours) after admission and then declined (Fig. 4.1). The plasma salicylic acid concentrations on admission and up to the time of peak concentration were measured by the ward side room assay and were in the range of 300 to 450 μg/ml. However, the values obtained were generally higher than those measured subsequently by high performance liquid chromatography (Fig. 2.8). The plasma salicylic acid half-lives were generally higher in these patients than in those in whom acetylsalicylic acid was not detected, ranging from 21 to 48 hours.

In another 7 patients in whom plasma acetylsalicylic acid was not detected after admission, absorption was apparently delayed (Fig. 4.1) and salicylic acid concentrations increased in one patient (No. 50) up to 16 hours. Two patients (Nos. 7 and 9) had also taken paracetamol plus d-propoxyphene with thyroxine and 2 had taken alcohol (Nos. 35 and 52). The plasma half-lives in this group ranged from 22 to 36 hours. Plasma salicylic acid concentrations in the other 5 patients are shown in Figure 4.2. The maximum plasma salicylic acid concentrations were observed on admission. The mean plasma salicylic acid half-life of all patients was 30.1 hours (Table 4.1).

Plasma /

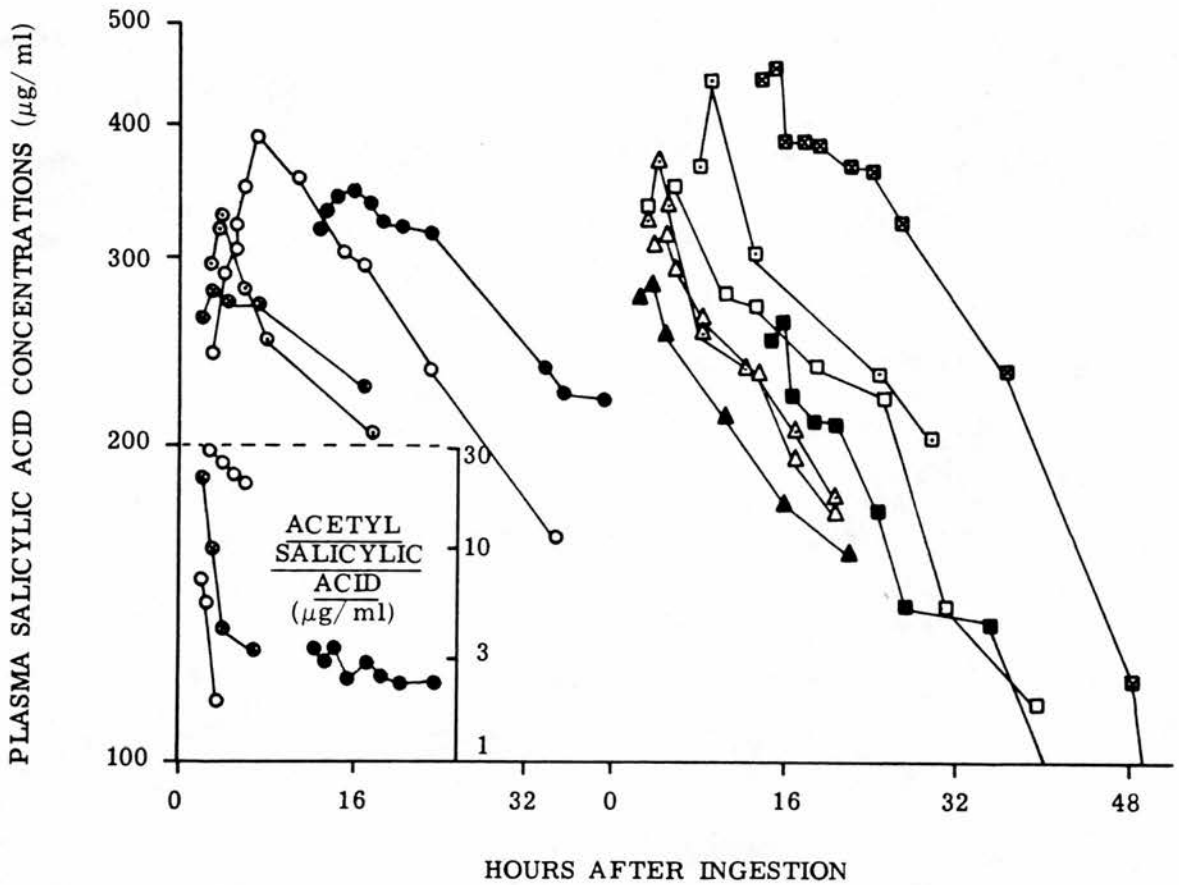

Figure 4.1. Plasma concentrations of salicylic acid in two groups of patients with mild aspirin poisoning who received oral fluids only showing delayed absorption. In the 4 cases (left) acetylsalicylic acid (inset, scale on right) was detected in the initial plasma samples.

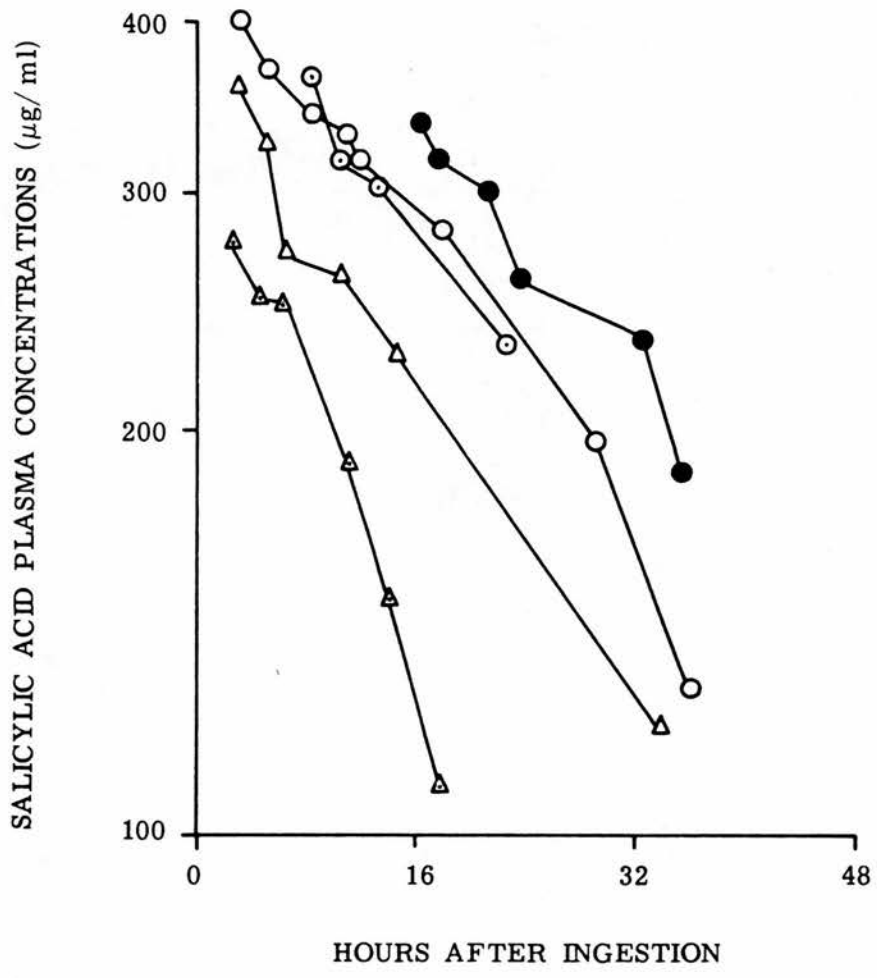

Figure 4.2. Plasma concentrations of salicylic acid in 5 patients with mild aspirin poisoning receiving oral fluids only.

TABLE 4.1. SALICYLIC ACID PHARMACOKINETICS IN PATIENTS WITH MILD ASPIRIN POISONING (CONTROLS)

Patient No.	Age and sex	Plasma half-life 4-16 hr	Total body clearance 4-16 hr (ml/min)	Apparent volume of distribution 4-16 hr (ml/kg)	Renal clearance (0-16 hr, ml/min)	Urinary recovery (0-16 hr, mg)
1	23 M	21.2	5.56	156	3.29	1035
4	21 F	20.3	5.94	180	1.90	169
6	29 F	32.6	9.02	374	3.22	638
7	44 F	20.6	5.57	184	2.44	558
8	16 F	26.3	N.A.*	N.A.	N.A.	N.A.
9	16 F	32.7	3.16	156	1.07	297
13	56 F	47.7	8.05	594	4.71	1061
16	62 F	24.7	4.96	204	0.58	84
34	19 F	23.2	4.84	102	1.18	315
35	21 M	23.3	8.0	217	0.24	116
36	21 F	34.1	2.73	132	0.21	371
43	24 M	29.5	2.80	111	0.15	51
45	34 M	45.7	3.38	203	0.40	384
48	42 F	40.7	5.12	396	0.42	242
50	17 F	22.9	6.79	328	0.38	88
52	38 M	35.7	5.22	197	0.41	234
Mean	30.2	30.1	5.41	236	1.37	376
S.D.	14.4	8.9	1.94	133	1.42	320

* not available due to incomplete urine collection

Plasma salicyluric acid concentrations were very low (mean 2.5-3.3 μg/ml) and remained relatively constant throughout the study (zero-order kinetics). The mean plasma salicyluric acid concentration in the control patients compared with the patients receiving intravenous treatment are shown in Figure 4.8.

Total body clearance and apparent volume of distribution of salicylic acid

The total body clearance and apparent volume of distribution of salicylic acid are shown in Table 4.1. Since the total body clearance was calculated from the renal clearance and urine collection was incomplete in patient No. 8, the total body clearance and thus volume of distribution of salicylic acid in this patient was not available. The total body clearance ranged from 2.7 to 9 ml/min and the apparent volume of distribution varied from 102 to 594 ml/kg. The patient who had the lowest volume distribution (102 ml/kg) was a 19 year old fat woman weighing 95.5 kg and the patient with the highest, had the longest plasma half-life due to possibly delayed and slow absorption.

The total body clearance of salicylic acid increased with time after 16 hours (> 10 ml/min).

Renal clearance and excretion of acetylsalicylic acid and its metabolites

The renal clearance of acetylsalicylic acid was only measurable in two patients (Nos. 1 and 13) and varied from 7 to 85 ml/min in different urine samples. Acetylsalicylic acid was only found in the urine of 6 patients and the mean recovery was 42.1 ± 27.3 mg in 4.6 ± 1.3 hours.

The /

The renal clearance and the urinary recovery of salicylic acid over the period 0-16 hours are shown in Table 4.1. The renal clearances ranged from 0.15 to 4.71 ml/min and the urinary recovery from 51 to 1061 mg. The mean renal clearance and urinary recovery of salicylic acid were 1.37 ml/min and 376 mg.

The renal clearance of salicyluric acid was much higher than the clearances of acetylsalicylic or salicylic acids. The mean was 493 ± 317 ml/min during the first 16 hours after admission (Fig. 4.9). The urinary recovery of salicyluric acid was much greater than that of salicylic acid in all patients (1116 ± 549 mg) except for one (No. 13). The urinary recoveries of salicyluric acid during the first 4 and 16 hours are given in Tables 4.19 and 4.20.

The urine pH did not change significantly during the study and the differences between patients were small with an overall mean of 6.1 ± 0.4. The urine flow rate varied from 0.58 to 3.03 ml/min with a mean of 1.40 ± 0.79 ml/min. The mean urine pH and flow rate over the first 4 and 16 hours are given in Table 4.9.

There was a highly significant correlation between the renal clearance of salicylic acid and urine pH ($r = 0.57$, $p < 0.001$) but no such correlation with salicyluric acid ($r = 0.185$, $p > 0.1$). There was also a highly significant correlation between the renal clearance of salicylic acid corrected for flow rate (the ratio of urine concentration to plasma concentration of salicylic acid) and urine pH. There was a negative correlation between the ratio of urine to plasma concentration of salicylic acid and urine flow rate ($r = -0.24$, $p < 0.05$).

Salicylic acid glucuronide conjugates accounted for 24% to 83% (59.6 ± 21.7) of the total urinary recovery of salicylic acid (Table 4.22). while /

while salicyluric acid glucuronide conjugates contributed 6% to 30% (14.3 ± 8.2) of the total urinary recovery of salicyluric acid (Table 4.23).

(d) Discussion

The absorption of acetylsalicylic acid was delayed in some patients after overdosage and in one it was detected as long as 24 hours after ingestion of 100 aspirin (Disprin) tablets. Since aspirin is not absorbed appreciably from the stomach, these findings indicate either slow dissolution or slow gastric emptying. This confirms the previous concept that gastric aspiration and lavage is never too late in aspirin poisoning (Matthew and Lawson, 1979).

Acetylsalicylic acid is rapidly hydrolysed to salicylic acid and only a small portion of the absorbed drug was excreted unchanged in the urine.

Salicylic acid is the major toxic metabolite of acetylsalicylic acid and since its binding to plasma proteins decreases significantly with increasing concentration (Chapter 3, Section II), disproportionately more unbound drug is available at higher concentrations to enter the tissues and induce toxicity.

Glycine and phenolic glucuronide conjugation of salicylic acid is saturated at low therapeutic concentrations (Levy, 1965a; Levy, Tsuchiya and Amsel, 1972), therefore its elimination through conjugation should have followed zero-order kinetics, although 32% of the salicylic acid was excreted unchanged. As a result the plasma half-life of salicylic acid was prolonged after overdosage to about 30 hours and was as long as 48 hours in one patient, whereas the mean in the healthy /

healthy subjects was 5.7 hours after an oral dose of 20 mg/kg (Chapter 1, Section III). This is consistent with the previous reports (Done, 1960; Levy and Yaffe, 1968).

The total body and renal clearances of salicylic acid were significantly lower in the overdose patients than the healthy volunteers given a therapeutic dose (Table 3.1). In the case of total body clearance this difference was due to saturation of glycine conjugation, the major route of elimination of a therapeutic dose. The increasing total body clearance of salicylic acid with time and decreasing plasma concentration is consistent with a change from zero-order to first-order elimination, although this could not be demonstrated in most patients. The higher apparent volume of distribution of salicylic acid following overdosage compared with the results in the healthy volunteers is in agreement with the dose-dependent volume of distribution found by Levy and Yaffe (1974). However, the apparent volume of distribution of non-protein bound salicylic acid declined over the concentration ranges found in patients with moderate to severe salicylate poisoning (Chapter 2, Section IV).

Renal clearance and excretion of salicylic acid is sensitive to urine pH and the correlation becomes greater when the renal clearance is corrected for flow rate. This is consistent with the results in healthy volunteers and the same explanation could be applied. The very high renal clearance of salicyluric acid was similar to that of the healthy volunteers and consistent with the findings of Levy et al. (1969). The mean urinary recovery of salicyluric acid was also greater than the recovery of salicylic acid, but more salicylic acid than /

than salicyluric acid glucuronide conjugates were excreted in the urine. This again is in agreement with the results in the healthy volunteers (Chapter 1, Section III) and previous reports (Rowland et al., 1967; Levy, 1978).

(e) Summary and conclusions

The pharmacokinetics of acetylsalicylic acid in overdosage were studied in 16 poisoned patients with plasma salicylate concentrations of 300-450 μg/ml.

Acetylsalicylic acid was slowly absorbed with delay in some patients. It was rapidly hydrolysed to salicylic acid and only a small fraction was excreted unchanged. The mean plasma half-life of salicylic acid was very prolonged with a mean value of 30 hours. The plasma concentration of salicyluric acid was very low and constant throughout reflecting saturation of glycine conjugation of salicylic acid.

There were direct relationships between the renal clearance of salicylic acid (but not salicyluric acid) and urine pH. The renal clearance of salicyluric acid exceeded the glomerular filtration rate and was much higher than that of salicylic acid due to active renal tubular secretion and conjugation.

The urinary recovery of salicyluric acid was greater than that of salicylic acid. On average, 65% was recovered as salicyluric acid, compared with 32% as salicylic acid.

SECTION IV

Chapter 2.

EFFECTS OF CHANGES IN URINE pH AND FLOW RATE ON THE PHARMACOKINETICS OF ACETYLSALICYLIC ACID FOLLOWING OVERDOSAGE

(a) Introduction

The effect of urine pH on the elimination of salicylate was studied many years ago (Hanzlik, 1926) and it was later confirmed that alkalinisation of the urine enhances salicylate elimination (Smith et al., 1946). The effects of intravenous sodium bicarbonate in children with salicylate poisoning (Oliver and Dayer, 1960) and the possible therapeutic usefulness of forced diuresis for the treatment of aspirin overdosage (Cumming, 1961) were reported.

Different methods of forced alkaline diuresis (Dukes et al., 1963; Cumming et al., 1964; Beveridge et al., 1964; Lawson et al., 1969; Savage et al., 1969),alkaline-mannitol diuresis (Prowse et al., 1970), mannitol-lactate and acetazolamide-bicarbonate (Morgan and Polak, 1969, 1971) and forced alkaline diuresis with loop diuretics (Berg, 1977) were reported. However, the separate effects of changes in urine pH and flow rate on the pharmacokinetics of acetylsalicylic acid have not been investigated in detail. In addition, problems such as delayed absorption, slow hydrolysis of acetylsalicylic acid, changes in salicylic acid distribution and the occasional failure to enhance salicylate elimination remain unsolved.

This /

This study was designed to investigate the pharmacokinetics of acetylsalicylic acid and the elimination of salicylate in patients with moderate to severe aspirin poisoning who were treated with one of four intravenous regimes of fluid and alkali. The study was approved by the Physicians' Advisory Ethical Committee of the Royal Infirmary, Edinburgh.

(b) Patients

Seventeen males and 17 females admitted to the Regional Poisoning Treatment Centre, Royal Infirmary, Edinburgh, with aspirin overdosage were studied. The patients were aged 16-65 years and their weights ranged from 43 - 86 kg. Patients with significant overdosage of other drugs or alcohol, respiratory, cardiac, renal, gastrointestinal and hepatic diseases or pregnancy were excluded.

The patients were divided into four groups, according to the admission or peak plasma salicylate concentrations (Chapter 1 of this Section) which were measured by the ward side room assay as follows :

1. Six patients (3 males and 3 females) with plasma salicylate concentrations of 400-550 µg/ml were treated by forced diuresis.
2. Six patients (3 males and 3 females) with plasma salicylate concentrations of 400-600 µg/ml were treated by alkaline diuresis.
3. Sixteen patients (8 males and 8 females) with plasma salicylate concentrations of 450-800 µg/ml were treated by forced alkaline diuresis.
4. Six patients (3 males and 3 females) with plasma salicylate concentrations of 400-700 µg/ml were treated by forced alkaline diuresis combined with frusemide.

(c) /

(c) Treatment regimes

The four intravenous regimes used were as follows :

1. Forced diuresis : One litre of 5% dextrose, 0.5 litre of normal saline and 0.5 litre of 0.6% (W/V) potassium chloride was made up in a glass bottle under sterile conditions. It was the standard "cocktail" for forced alkaline diuresis without the sodium bicarbonate and was infused at a rate of 2 litres per hour for 3 hours.

2. Alkali alone : 1.26% ($^{M}/_{6}$) sodium bicarbonate solution containing 1.5 g potassium chloride in each 0.5 litre was given intravenously at a rate of 0.5 litre per hour for 3 hours.

3. Forced alkaline diuresis : One litre of 5% dextrose, 0.5 litre of normal saline and 0.5 litre of 1.26% sodium bicarbonate plus 3 g potassium chloride was infused at a rate of 2 litres per hour for 3 hours. This was the standard "cocktail" forced alkaline diuresis and thus the same as regime 1 with the addition of 6.3 g sodium bicarbonate to each 2-litre bottle. The sodium bicarbonate was added just before use as 75 ml of 8.4% sodium bicarbonate to 1925 ml solution already in the bottle.

4. Forced alkaline diuresis with frusemide : The same regime as the forced alkaline diuresis was used with the intravenous injection of 40 mg frusemide at the end of the first 2 litres and a further 2 doses of 20 mg each at the end of the 4 th and 6th litres.

(d) Titration of solutions used for the forced diuresis and forced alkaline diuresis

Twenty ml of the solution (cocktail) used for forced diuresis was titrated against 0.001 N sodium bicarbonate using a pH M 62 standard pH meter (Radiometer, Copenhagen). The solution (cocktail) used /

used for forced alkaline diuresis (20 ml) was titrated against 0.01 N hydrochloric acid. These titrations were performed on five randomly selected samples of the two types of cocktail solution. The results are summarised in Table 4.2. and a titration curve of each is shown in Figure 4.3. There were no significant differences between the samples. The buffer capacity of the solution used for forced alkaline diuresis was much greater than the solution used for forced diuresis.

(e) Methods

Blood and urine sample collections and analyses were as described for the control patients (Chapter 1, Section IV) except for:

1. Blood sampling times : On admission, at the commencement of the infusion, hourly for 5 hours and then at 7, 11, 15, 23, 35 and 47 hours after the start of intravenous therapy.
2. In 7 patients including 2 controls, the binding of salicylic acid to plasma proteins was measured in freshly taken samples.
3. The total urinary output of salicylic acid including glucuronide conjugates of salicylic and salicyluric acids was measured in 6 patients from each group (except for forced alkaline diuresis with frusemide).
4. The volumes of distribution of the total and unbound salicylic acid were measured by the method of Levy and Yaffe (1974) (i.e. total body salicylate divided by the plasma concentration of total/unbound salicylate) in five patients who were kept in the ward until salicylate was no longer detectable in the urine.
5. In all the patients, including the controls, the apparent plasma half-life of salicylic acid was calculated from the time of the peak plasma /

TABLE 4.2. TITRATION OF SOLUTIONS (20 ml) USED FOR FORCED DIURESIS AND FORCED ALKALINE DIURESIS

	Solution No.							Coefficient of variation
	1	2	3	4	5	Mean	S.D.	%
pH of solutions used for forced diuresis	3.92	3.97	4.13	4.05	3.90	3.99	0.09	2.2
Volume of 0.001 N sodium bicarbonate required to reach pH 7.0 (ml)	11.7	10	10	10	12	10.74	1.02	9.50
Volume of 0.001 N sodium bicarbonate required to reach pH 8.5 (ml)	15.2	15.0	14.8	15.0	15.7	15.14	0.34	2.25
pH of solutions used for forced alkaline diuresis	9.14	9.20	9.25	9.20	9.19	9.20	0.04	0.4
Volume of 0.01 N hydrochloric acid required to reach pH 7.0 (ml)	25.5	25.0	25.0	22.0	22.0	24.0	1.75	7.3
Volume of 0.01 N hydrochloric acid required to reach pH 6.0 (ml)	75	65	65	60	62	65.40	5.8	8.9

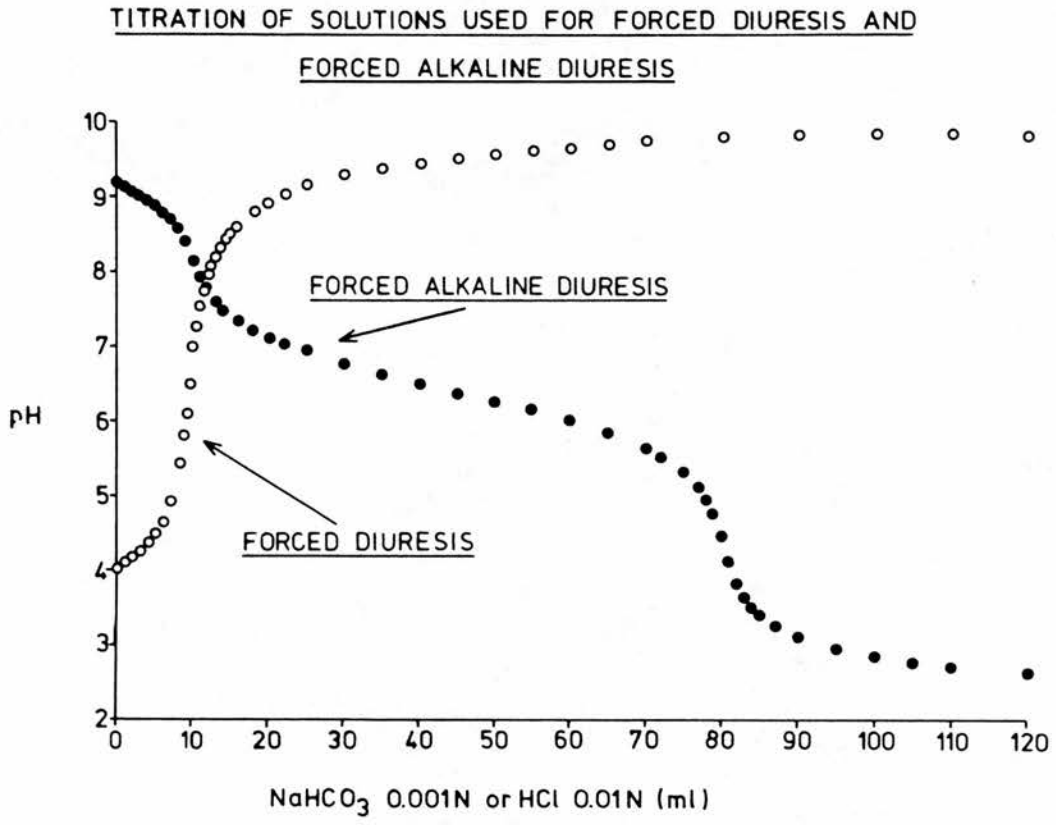

Figure 4.3. Titration of 20 ml of the solution used for forced diuresis against 0.001 N sodium bicarbonate, and 20 ml of the solution used for forced alkaline diuresis against 0.01 N hydrochloric acid.

plasma concentration up to 4 hours and from 4 to 16 hours. The total body clearance and apparent volume of distribution of salicylic acid were also calculated over these two time periods.

6. The renal clearances and urinary recoveries of salicylic and salicyluric acids were calculated over the periods 0-4 and 0-16 hrs.

7. Comparisons within and between the groups were performed using the one way analysis of variance. If the differences were significant ($p < 0.05$), individual groups were compared using the independent, two-tailed Student t-test.

8. Multiple regression analysis (Woolf, 1951) was used to evaluate the separate effect of urine pH and flow rate on the ratio of urine to plasma salicylic acid concentrations.

(f) Results

Plasma concentrations and elimination of acetylsalicylic acid and its metabolites

Acetylsalicylic acid

Acetylsalicylic acid was detected in the plasma on admission in 4, 5, 7 and 4 patients with forced diuresis, alkali alone, forced alkaline diuresis and forced alkaline diuresis with frusemide respectively. The concentrations varied from 3.1 µg/ml to 37.4 µg/ml and the means for each group were 10.2 ± 7.0, 15.4 ± 14.0, 11.4 ± 7.2 and 5.7 ± 1.8 µg/ml respectively. Plasma acetylsalicylic acid concentrations rose following admission in one patient (No. 29) with forced diuresis, one (No. 17) with forced alkaline diuresis and two (Nos. 54 and 59) with forced alkaline diuresis plus frusemide. Serial plasma acetylsalicylic acid concentrations in each group /

group including the controls are shown in Figure 4.4. The plasma acetylsalicylic acid concentration in patient No. 17 increased up to 39.7 μg/ml at 6¾ hours in spite of infusion of 2 litres of fluid and this accounts for the higher mean concentration at 2 hours after admission in the forced alkaline diuresis group.

It was not possible to measure the plasma acetylsalicylic acid half-life in most patients because absorption was apparently delayed and variable.

Salicylic acid

The mean and standard deviations of serial plasma concentrations of salicylic acid are given in Table 4.3. The interval between the reported time of ingestion and the admission plasma sample varied from 1 - 24 hours with a mean of 9.5, 6.9, 6.8, 3.6 and 3.5 hours in the forced alkaline diuresis, forced alkaline diuresis plus frusemide, control, alkali alone and forced diuresis groups, respectively. The mean plasma salicylic acid concentrations on admission were 497 ± 87, 472 ± 63, 471 ± 84, 439 ± 86 and 328 ± 57 μg/ml for the forced alkaline diuresis, forced alkaline diuresis with frusemide, forced diuresis, alkali alone and control groups, respectively. The plasma concentrations on admission were significantly higher in the treated groups than in the control patients ($p < 0.005$). A late rise in plasma salicylic acid concentrations was only observed in two cases, presumably because of the lowering effects of treatment. The mean plasma salicylic acid concentrations for all groups from the time of admission up to 16 hours are shown in Figure 4.5.

During infusion (0-4 hours) /

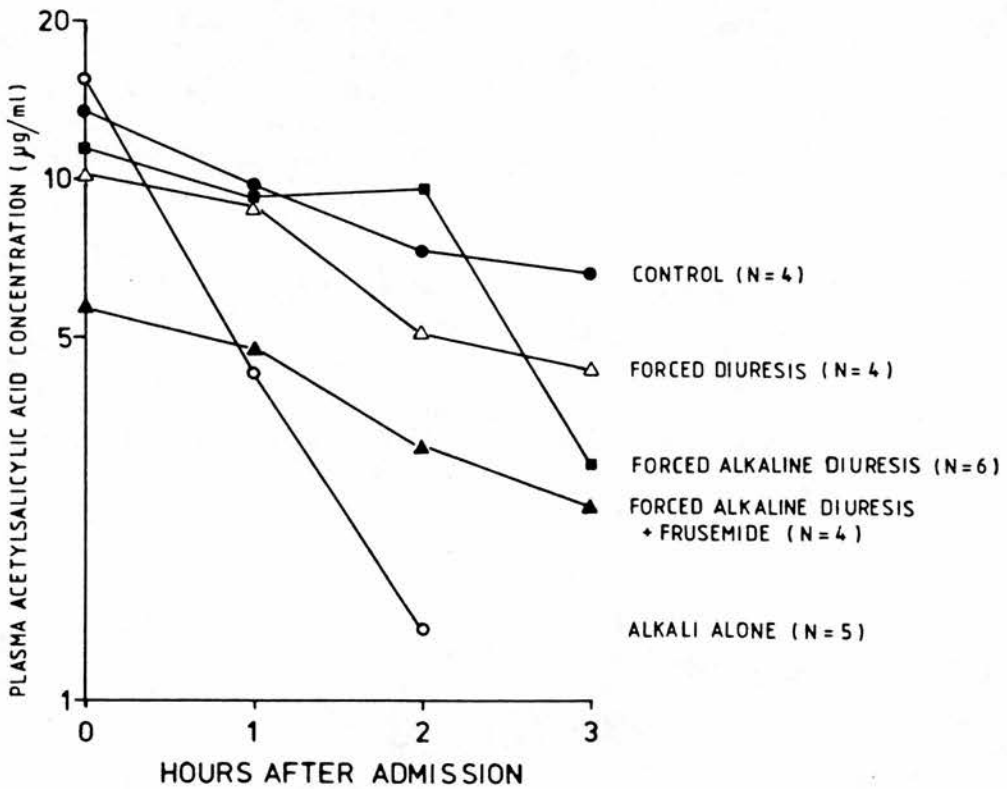

Figure 4.4. Mean plasma concentrations of acetylsalicylic acid in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

TABLE 4.3. PLASMA CONCENTRATIONS OF SALICYLIC ACID (μg/ml)

		Ingestion* admission interval	On admission	Hours 1	2	3	4	5	6	8	12	16
Control	Mean	6.83	328	337	317	304	299	294	284	270	249	223
	S.D.	5.10	57	53	46	47	50	52	50	54	50	45
Forced alkaline diuresis	Mean	9.50	497	454	399	338	285	260	241	204	154	119
	S.D.	5.45	87	108	103	101	101	102	101	98	95	95
FAD + frusemide	Mean	6.92	472	457	423	394	341	307	290	260	215	165
	S.D.	8.71	63	70	59	60	42	42	41	51	47	61
Forced diuresis	Mean	3.46	471	445	387	362	315	310	302	290	266	224
	S.D.	1.59	84	54	56	70	69	72	72	69	57	54
Alkali alone	Mean	3.62	439	414	389	339	286	253	227	191	134	100
	S.D.	3.77	86	74	93	90	90	86	77	85	83	77

* Interval between reported ingestion time and on admission plasma sample

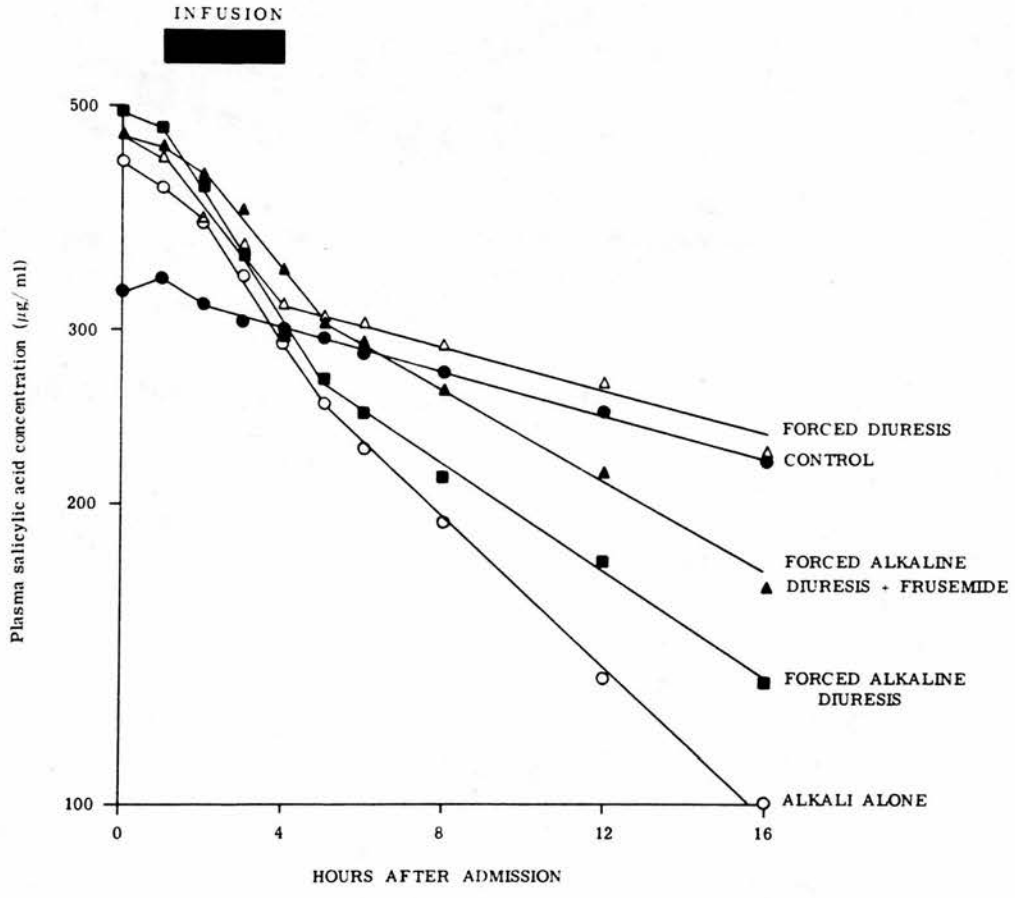

Figure 4.5. Mean plasma concentrations of salicylic acid in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

During infusion (0-4 hours)

The mean plasma salicylic acid concentrations increased after admission in the control group, possibly due to delayed absorption and or slow hydrolysis of acetylsalicylic acid. The mean plasma salicylic acid concentrations declined during the infusion in all treated groups. The apparent plasma half-life values of salicylic acid from 0 to 4 hours in each patient including the controls are given in Table 4.4. The mean plasma half-lives were 5.05, 6.37, 6.58, 8.54 and 19.4 hours with alkali alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis and control groups, respectively. The plasma half-life of salicylic acid was significantly longer with the control than in the treated groups ($p < 0.001$). There were no statistically significant differences between the plasma salicylic acid half-lives of the alkalinisation groups. However, it was significantly longer with the forced diuresis than in the alkali alone group ($p < 0.02$), but not the other alkalinisation groups.

After infusion (4-16 hours)

The rate of disappearance of salicylic acid from the plasma during the 4-16 hour period was the same in the forced diuresis and control groups (30.6 ± 5.7 and 30.1 ± 8.8 respectively). The plasma half-life values of salicylic acid from 4 to 16 hours in each patient are given in Table 4.5. The mean plasma half-lives in the alkalinisation groups were 9.14, 11.6 and 14.1 with alkali alone, forced alkaline diuresis and forced alkaline diuresis plus frusemide, respectively. There were no statistically significant differences /

TABLE 4.4.

PLASMA HALF-LIFE OF SALICYLIC ACID 0-4 HOURS (hr)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	hr	No.	hr	No.	hr	No.	hr	No.	hr
1	25.6	2	3.15	53	5.85	27	11.8	37	5.27
4	12.5	3	7.64	54	6.32	29	7.43	38	5.63
6	10.7	5	6.32	55	4.20	30	N.M.	44	4.50
7	13.7	10	7.59	57	5.01	31	9.92	47	6.94
8	16.3	14	3.32	58	6.74	32	5.56	49	3.32
9	7.4	15	5.91	59	10.1	33	7.97	51	4.62
13	47.7	17	18.5						
16	6.9	19	N.M.*						
34	7.5	20	4.24						
35	16.3	21	5.59						
36	25.6	22	5.21						
43	15.6	23	3.27						
45	45.7	24	11.8						
48	21.6	25	3.74						
50	14.2	26	3.47						
52	23.8	41	8.92						
Mean:	19.4		6.58		6.37		8.54		5.05
S.D.:	12.2		4.11		2.04		2.40		1.22

Analysis of variance: F-Ratio = 7.02 $p < 0.01$

* not measurable

TABLE 4.5.

PLASMA HALF-LIFE OF SALICYLIC ACID 4-16 HOURS (hr)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	hr	No.	hr	No.	hr	No.	hr	No.	hr
1	21.2	2	4.48	53	5.93	27	39.2	37	9.11
4	20.3	3	11.3	54	13.0	29	32.2	38	18.9
6	32.6	5	9.74	55	27.2	30	N.M.	44	6.24
7	20.6	10	18.7	57	6.15	31	26.0	47	11.6
8	26.3	14	7.50	58	15.5	32	24.8	49	2.62
9	32.7	15	5.11	59	16.9	33	30.6	51	4.86
13	47.7	17	7.86						
16	24.7	19	N.M.*						
34	23.2	20	9.77						
35	23.3	21	N.M.						
36	34.1	22	4.97						
43	29.5	23	N.M.						
45	45.7	24	35.8						
48	40.7	25	7.10						
50	22.9	26	5.82						
52	35.7	41	22.10						
Mean :	30.1		11.6		14.1		30.6		9.14
S.D. :	8.8		9.01		6.7		5.73		5.88

Analysis of variance : F-Ratio = 15.6 $p < 0.01$

* not measurable

differences between the plasma salicylic acid half-lives of the alkalinisation groups. However, the plasma half-life values were significantly shorter with the alkalinisation groups than in the forced diuresis and control groups ($p < 0.001$).

Plasma half-life of salicylic acid could not be measured in patients Nos. 19 and 30 because of delayed absorption and infusion of 8 litres of fluid in the former and incomplete plasma collection in the latter.

Since the plasma concentrations of salicylic acid on admission were not the same in all groups, subsequent values were expressed as the percentages of the initial plasma concentrations and are shown in Figure 4.6.

There was a significant negative correlation between the plasma half-life of salicylic acid from 4-16 hours and the highest urine pH measured in that period ($r = -0.80$, $p < 0.001$) as shown in Figure 4.7. The patients treated with alkali alone (●) had higher urine pH and shorter plasma half-life of salicylic acid, whereas the control patients (o) and those treated with forced diuresis (⊕) had lower urine pH and longer plasma half-life than the other groups.

Salicyluric acid

Plasma concentrations of salicyluric acid were very low in all patients throughout the study, ranging from 0.5 to 10 μg/ml. The mean plasma concentration curves of salicyluric acid are shown in Figure 4.8. Although the mean plasma concentrations of salicyluric acid were higher (not statistically significant) in the forced alkaline diuresis with frusemide group, there were no statistically significant /

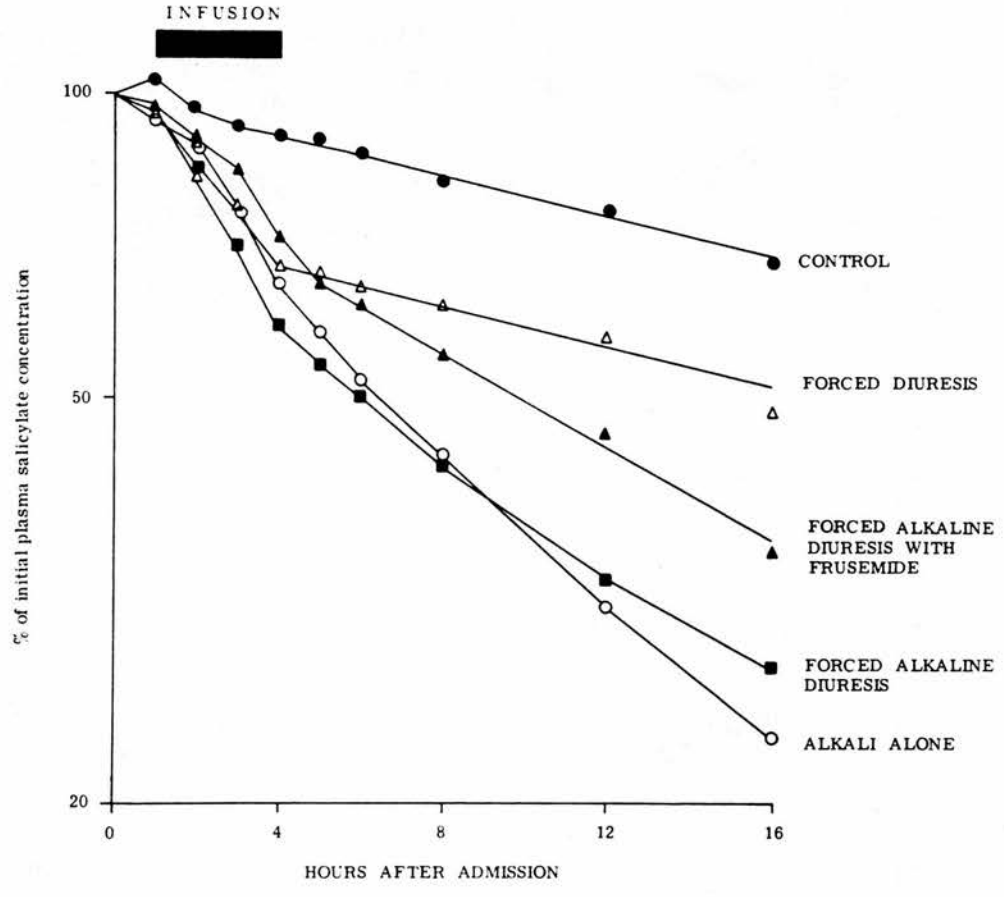

Figure 4.6. Plasma concentrations of salicylic acid expressed as percentage of initial concentrations in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

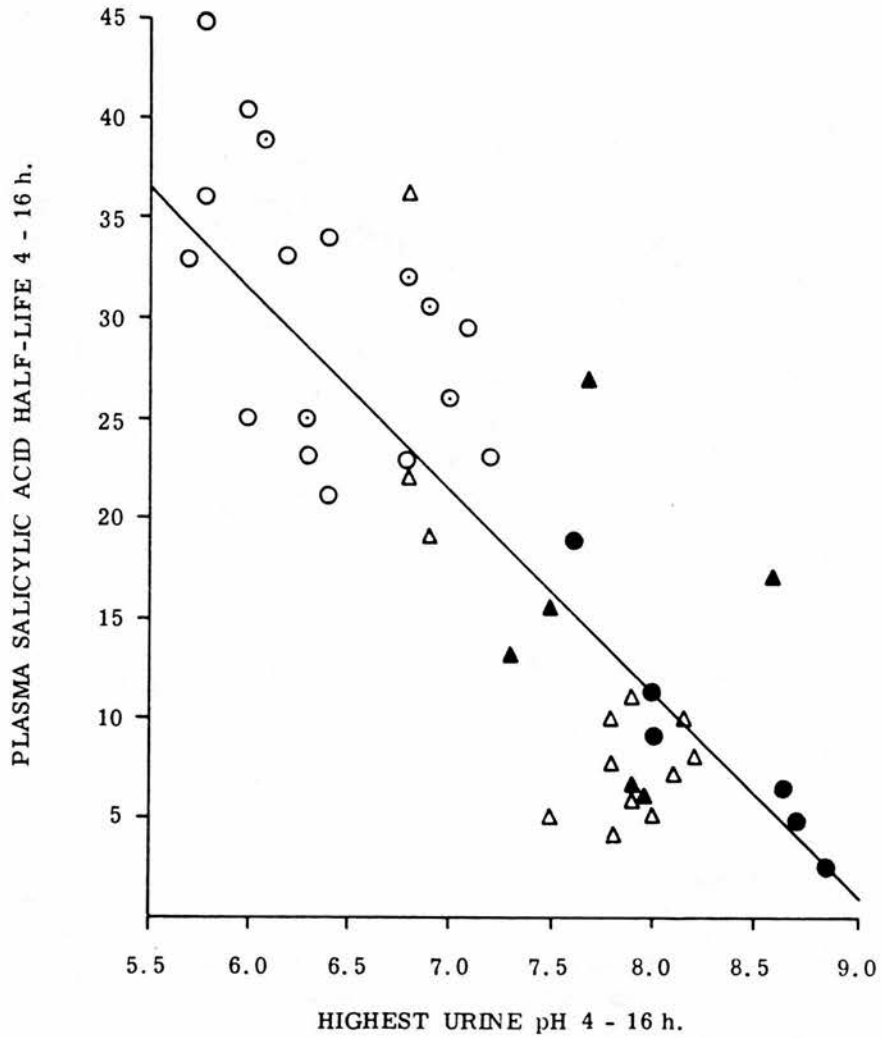

Figure 4.7. Correlation between the plasma half-life of salicylic acid after infusion and the maximum urine pH over that period. ○ control, ⊙ forced diuresis, △ forced alkaline diuresis, ▲ forced alkaline diuresis with frusemide, ● alkali alone.

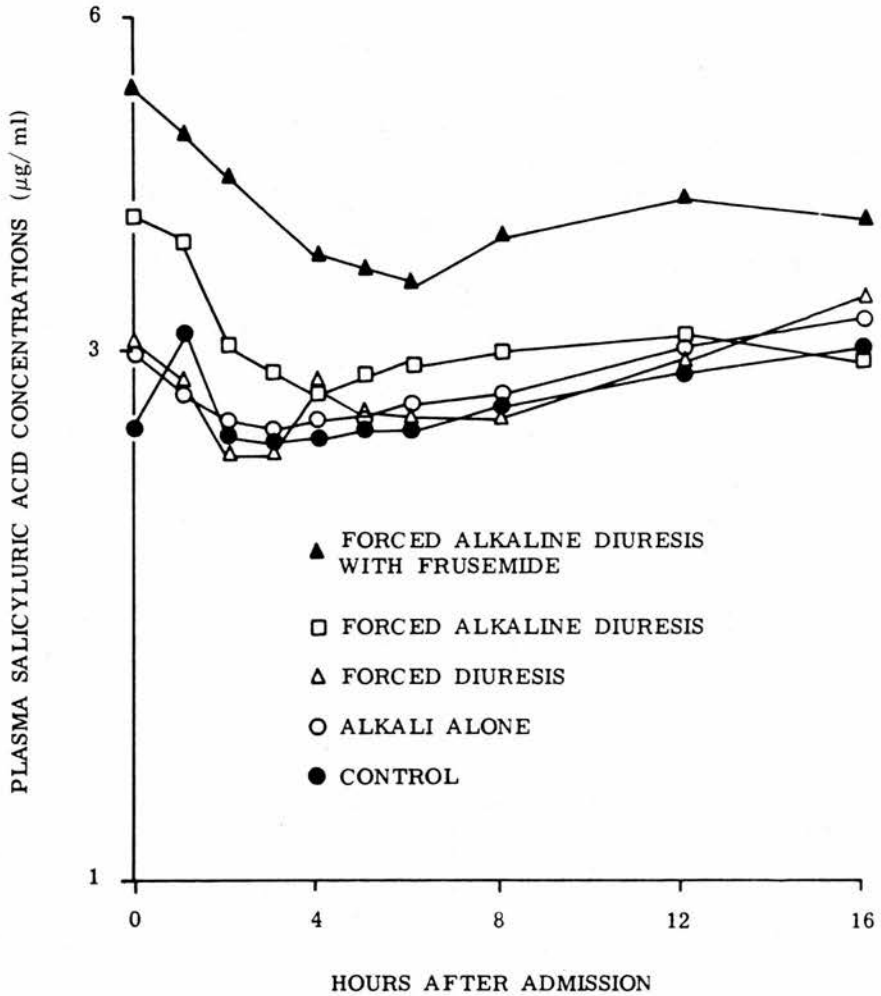

Figure 4.8. Mean plasma concentrations of salicyluric acid in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

significant differences between the areas under the plasma salicyluric acid concentration-time curves of any of the groups.

The plasma salicyluric acid concentrations in the treated groups fell during the infusion (0-4 hr). The percentage fall was 30, 29, 20 and 14 with forced alkaline diuresis, forced alkaline diuresis with frusemide, forced diuresis and alkali alone. respectively. These were all significantly higher than the 2% fall in the control group ($p < 0.001$). The fall was significantly higher in the forced alkaline diuresis than in the alkali alone group ($p < 0.01$).

Total body clearance and apparent volume of distribution of salicylic acid

The total body clearances and apparent volumes of distribution of salicylic acid during the 0-4 and 4-16 hour periods are shown in Table 4.6. In the control group the mean total body clearance was slightly higher during the second period (4.90 ± 2.90 and 5.41 ± 1.94 respectively) and it appeared to increase with time. As the plasma concentration of salicylic acid declined below 100 µg/ml it increased more than 3 fold (above 18 ml/min). In the other groups, however, the total body clearances of salicylic acid declined after the infusion. The mean percentage decreases were 63, 60, 45 and 36 with forced diuresis, forced alkaline diuresis with frusemide, forced alkaline diuresis and alkali alone groups, respectively. The mean total body clearances of salicylic acid were 18.8, 36.8, 36.9 and 37.5 during, and 6.9, 14.7, 20.3 and 24 ml/min after infusion respectively. These values were significantly higher than in the control group, particularly during the infusion period ($p < 0.001$ and $p < 0.01$ between the forced diuresis and control groups during and after infusion /

TABLE 4.6. TOTAL BODY CLEARANCE AND APPARENT VOLUME OF DISTRIBUTION OF SALICYLIC ACID

Group	Total body clearance (ml/min)		Volume of distribution (ml/kg)	
	0-4 hr	4-16 hr	0-4 hr	4-16 hr
Control	4.90 ± 2.90	5.41 ± 1.94	131 ± 76	236 ± 133
Forced diuresis	18.8 ± 12.2	6.90 ± 1.93	217 ± 141	285 ± 80
Alkali alone	37.5 ± 10.9	24.0 ± 14.9	276 ± 109	319 ± 198
Forced alkaline diuresis	36.9 ± 13.3	20.3 ± 9.7	355 ± 128	348 ± 166
Forced alkaline diuresis with frusemide	36.8 ± 10.0	14.7 ± 6.83	330 ± 90	292 ± 135

Values are given as mean ± standard deviations

infusion respectively). There were no statistically significant differences in the total body clearance of salicylic acid in the alkalinisation groups, but the clearance values were significantly higher in the alkalinisation than in forced diuresis groups ($p < 0.05$ during and $p < 0.01$ after infusion).

The mean apparent volumes of distribution of salicylic acid during the infusion (0-4 hours) were 355, 330, 276, 217 and 131 ml/kg in the forced alkaline diuresis, forced alkaline diuresis plus frusemide, alkali alone, forced diuresis and control groups respectively. The values increased after 4 hours in the control, forced diuresis and alkali alone groups, and decreased in the other 2 groups, but the changes were not statistically significant. The mean apparent volume of distribution of salicylic acid at 4-16 hrs was 348, 319, 292, 285 and 236 ml/kg in the forced alkaline diuresis, alkali alone, forced alkaline diuresis plus frusemide, forced diuresis and control groups, respectively. There were no statistically significant differences in the apparent volume of distribution of salicylic acid between the groups.

The apparent volumes of distribution of salicylic acid increased significantly with concentration in all patients except No. 57 in whom acute on chronic salicylate intoxication occurred. The volumes of distribution did not change significantly with plasma salicylic acid concentrations in this patient (Table 4.7.).

Total body clearance and apparent volume of distribution of unbound salicylic acid /

TABLE 4.7. APPARENT VOLUME OF DISTRIBUTION OF UNBOUND (U) AND TOTAL (T) SALICYLIC ACID (ml/kg) RELATED TO PLASMA CONCENTRATIONS (P) OF TOTAL SALICYLIC ACID (μg/ml)

Patient : No. 25			Patient : No. 54			Patient : No. 57			Patient : No. 44			Patient : No. 49		
U	T	P	U	T	P	U	T	P	U	T	P	U	T	P
1070	166	95	285	98	237	1427	178	75	1107	210	120	2102	200	88
866	182	136	265	119	312	792	155	125	910	214	154	771	254	224
719	202	180	296	148	350	513	136	178	646	226	242	579	272	328
643	209	220	322	164	365	395	138	240	528	251	335			
584	213	250	312	176	400	366	141	265	460	276	430			
429	197	320	307	187	436	376	150	278						
			366	227	441	347	149	300						
						323	148	320						
						282	142	354						
						266	142	376						

Total body clearance and apparent volume of distribution of unbound salicylic acid

The total body clearance and apparent volume of distribution of unbound salicylic acid in the 5 patients are given in Table 4.8. The mean values from 0-4 hours were 78 ml/min and 469 ml/kg which were significantly greater than the corresponding total body clearance and apparent volume of distribution of total salicylic acid ($p < 0.01$). Both the total body clearance and apparent volume of distribution of unbound salicylic acid significantly increased after infusion ($p < 0.01$) and the mean values (4-16 hours) were 151 ml/min and 1148 ml/kg. These were much higher than the corresponding total body clearance and apparent volume of distribution of total salicylic acid ($p < 0.001$).

The apparent volume of distribution of unbound salicylic acid decreased with increasing plasma concentration of the total drug in all patients except in patient No. 54 in which it did not change significantly (Table 4.7.). However, there was a significant overall negative correlation between the apparent volumes of distribution of unbound salicylic acid and the plasma concentrations of the total drug ($r = -0.77$, $p < 0.001$).

Urinary pH and flow rate

The mean and standard deviations of urine pH and flow rate over the first 4 and 16 hours after admission for each group of patients are given in Table 4.9. The range of urine pH in each patient was usually small, except for the alkalinisation groups in which pH was higher during and for a variable period after the infusion. /

TABLE 4.8.

TOTAL BODY CLEARANCE AND APPARENT VOLUME OF DISTRIBUTION OF UNBOUND SALICYLIC ACID

Group	No.	Total body clearance (ml/min)		Volume of distribution (ml/kg)	
		0-4 hr	4-16 hr	0-4 hr	4-16 hr
Forced alkaline diuresis	25	120	161	594	1514
Forced alkaline diuresis with frusemide	54	41	61	343	1037
	57	52	115	336	911
Alkali alone	44	69	90	494	888
	49	108	328	579	1392
Mean		78	151	469	1148
S.D.		35	105	124	287
P - value		< 0.01		< 0.01	

TABLE 4.9.

URINE pH AND FLOW RATE IN PATIENTS WITH ASPIRIN OVERDOSAGE

Group	Urine pH		Urine flow rate (*ml/min*)	
	0-4 hr	0-16 hr	0-4 hr	0-16 hr
Control	6.4 ± 0.5	6.1 ± 0.4	2.25 ± 2.14	1.40 ± 0.79
Forced alkaline diuresis (FAD)	7.6 ± 0.3	7.4 ± 0.4	15.5 ± 6.9	5.05 ± 1.75
F A D plus frusemide	7.6 ± 0.5	7.5 ± 0.4	24.8 ± 4.3	6.46 ± 1.15
Forced diuresis	7.1 ± 0.6	6.5 ± 0.3	8.18 ± 4.49	5.80 ± 1.88
Alkali alone	8.2 ± 0.5	8.1 ± 0.5	7.00 ± 4.50	2.60 ± 0.70

infusion. The mean urine pH from 0-4 hrs was 8.2, 7.6, 7.6, 7.1 and 6.4, and from 0-16 hrs 8.1, 7.5, 7.4, 6.5 and 6.1 in the alkalie alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis and control groups respectively. There were no significant differences between the period of infusion and the 12 hours after in the treated groups, but there were differences between the alkalinisation regimes and the forced diuresis or control groups ($p < 0.001$). Alkali alone gave the highest urine pH (0-16 hours) which was significantly higher than with the forced alkaline diuresis ($p < 0.01$), but not forced alkaline diuresis with frusemide. There were no significant differences between the urine pH of the forced diuresis and control groups.

The urine flow rates were significantly increased with forced diuresis (with and without alkali), although the onset of diuresis was often delayed. There was no delay in the patients who received frusemide. The mean urine flow rates from 0-4 hours were 25,15, 8.2, 7.0 and 2.25; and from 0-16 hours 6.46, 5.05, 5.80, 2.60 and 1.40 ml/min with forced alkaline diuresis plus frusemide, forced diuresis, forced alkaline diuresis, alkali alone and the controls respectively. There were highly significant differences between the urine flow rates (0-16 hours) of the diuresis regimes and the control or alkali alone groups ($p < 0.001$). The urine flow rates of the alkali alone group were also greater than those of the controls ($p < 0.005$).

Renal clearance of acetylsalicylic acid

The renal clearances of acetylsalicylic acid could only be measured in the first few urine samples from 10 patients (6 with forced /

forced alkaline diuresis, 2 controls, one with alkali alone and one with forced alkaline diuresis plus frusemide). The renal clearances ranged from 11.7 ml/min in a control patient (No. 1) to 343 ml/min in a patient who received forced alkaline diuresis with frusemide. Another patient (No. 23) given forced alkaline diuresis also had a very high renal clearance of acetylsalicylic acid of 333 ml/min. The overall mean renal clearance of acetylsalicylic acid was 110 ± 123 ml/min. The correlations between the renal clearance of acetylsalicylic acid and urine pH or flow rate (using individual urine samples) either corrected or uncorrected for flow rate were not statistically significant.

Renal clearance of salicylic acid

The renal clearances of salicylic acid of all patients for the first 4 and 16 hours after admission are shown in Tables 4.10 and 4.11. The mean renal clearances from 0-4 hours were 31, 22, 20, 4.4 and 2.2 ml/min in the alkali alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis and control groups, respectively. The renal clearances of salicylic acid decreased significantly after the infusion only in the forced alkaline diuresis with frusemide group ($p < 0.01$). The mean renal clearances from 0-16 hours were 23.5, 17.5, 12.7, 4.40 and 1.37 in the alkali alone, forced alkaline diuresis, forced alkaline diuresis plus frusemide, forced diuresis and control groups respectively.

The renal clearances of salicylic acid over both periods were significantly higher with the alkalinisation groups than in the forced /

TABLE 4.10.

RENAL CLEARANCE OF SALICYLIC ACID 0-4 HOURS (ml/min)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min
1	3.69	2	50.0	53	19.2	27	3.12	37	13.6
4	3.60	3	14.5	54	26.7	29	2.30	38	18.4
6	2.10	5	16.5	55	20.8	30	0.65	44	19.0
7	4.52	10	17.2	57	6.31	31	12.5	47	24.0
8	N.A.*	14	9.60	58	32.8	32	4.62	49	26.3
9	1.85	15	10.0	59	23.6	33	3.00	51	85.7
13	8.00	17	13.7						
16	0.23	19	37.1						
34	1.17	20	17.4						
35	0.72	21	44.7						
36	1.33	22	8.22						
43	0.20	23	18.1						
45	1.30	24	2.75						
48	1.40	25	19.3						
50	0.65	26	37.6						
52	2.03	41	3.08						
Mean:	2.20		20.0		21.6		4.37		31.2
S.D.:	2.05		14.5		8.9		4.20		27.1

Analysis of variance: F - ratio = 8.0 $p < 0.01$

* not available

TABLE 4.11.

RENAL CLEARANCE OF SALICYLIC ACID 0-16 HOURS (ml/min)

Control		Forced alkaline diuresis(FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min
1	3.29	2	42.4	53	12.8	27	2.43	37	18.0
4	1.90	3	6.7	54	14.8	29	3.56	38	10.2
6	3.22	5	22.0	55	13.2	30	4.04	44	28.4
7	2.44	10	15.3	57	7.5	31	6.79	47	16.4
8	N.A.*	14	27.7	58	14.8	32	7.18	49	48.7
9	1.07	15	N.A.	59	13.3	33	2.41	51	19.5
13	4.71	17	13.3						
16	0.58	19	20.4						
34	1.18	20	17.8						
35	0.24	21	N.A.						
36	0.21	22	19.2						
43	0.15	23	N.A.						
45	0.40	24	9.2						
48	0.42	25	23.4						
50	0.38	26	17.9						
52	0.41	41	4.9						
Mean:	1.37		17.5		12.7		4.40		23.5
S.D.:	1.42		10.1		2.3		1.78		13.7

Analysis of variance: F - Ratio = 14.895 $p < 0.01$

* not available

forced diuresis or control groups ($p < 0.001$). There were no statistically significant differences between the three alkalinisation groups, but there was a significant difference in the renal clearance of salicylic acid 0-16 hours between the forced diuresis and control groups ($p < 0.005$).

Renal clearance of unbound salicylic acid

The renal clearances of unbound salicylic acid of the 7 patients in which measurements were made ranged from 0.65 ml/min in a control patient (No. 52) to 172 ml/min in a patient (No. 49) treated with alkali alone (Table 4.12). Two other patients (Nos. 54 and 58) who received forced alkaline diuresis with frusemide also had renal clearances of free salicylic acid above 100 ml/min in the individual urine samples (118 and 125 ml/min respectively). Although the renal clearances of unbound salicylic acid were much less in the control patients (Nos. 50 and 52) than the other 5 treated patients, they were greater than the corresponding total salicylic acid renal clearances.

Renal clearance of salicyluric acid

The renal clearances of salicyluric acid from 0-4 and from 0-16 hrs after admission are shown in Tables 4.13 and 4.14. The renal clearances of salicyluric acid were much greater than those of salicylic acid /

TABLE 4.12.

RENAL CLEARANCE OF UNBOUND SALICYLIC ACID

Group	No.	Renal clearance (ml/min)	
		0 - 4 hr	4 - 16 hr
Control	50	1.11	1.50
	52	3.71	0.65
Forced alkaline diuresis	25	54	34
Forced alkaline diuresis with frusemide	54	69	11
	58	69	40
Alkali alone	44	68	63
	49	132	172
Mean		57	46
S.D.		45	60

TABLE 4.13.

RENAL CLEARANCE OF SALICYLURIC ACID 0-4 HOURS (ml/min)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min
1	270	2	625	53	773	27	855	37	459
4	422	3	317	54	460	29	191	38	654
6	261	5	398	55	343	30	778	44	342
7	420	10	413	57	285	31	602	47	474
8	N.A.*	14	484	58	1126	32	881	49	798
9	256	15	463	59	273	33	208	51	1037
13	518	17	539						
16	457	19	568						
34	241	20	305						
35	450	21	908						
36	308	22	351						
43	387	23	218						
45	189	24	461						
48	598	25	695						
50	902	26	987						
52	1110	41	375						
Mean:	453		507		543		586		627
S.D.:	255		212		340		315		257

Analysis of variance: F - Ratio = 0.62 $p > 0.05$

* not available

TABLE 4.14.

RENAL CLEARANCE OF SALICYLURIC ACID 0-16 HOURS (ml/min)

Control		Forced alkaline diuresis(FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min
1	318	2	411	53	583	27	740	37	309
4	329	3	410	54	207	29	265	38	415
6	955	5	508	55	463	30	589	44	255
7	246	10	352	57	492	31	264	47	292
8	N.A.*	14	476	58	375	32	323	49	1067
9	183	15	523	59	217	33	258	51	992
13	376	17	485						
16	265	19	298						
34	294	20	360						
35	426	21	N.A.						
36	403	22	383						
43	422	23	N.A.						
45	300	24	367						
48	986	25	648						
50	1231	26	482						
52	663	41	263						
Mean:	493		426		390		407		555
S.D.:	317		101		153		207		37.2

Analysis of variance : F - Ratio = 1.23 $p > 0.05$

* not available

acid, ranging from 183 ml/min in patient No. 9 to 1231 ml/min in patient No. 50 - both were in the control group (0-16 hours). The mean values from 0-16 hours were 555, 493, 426, 407 and 390 ml/min with alkali alone, control group, forced alkaline diuresis, forced diuresis and forced alkaline diuresis plus frusemide respectively. There were no significant differences between the groups ($p > 0.05$). Although the renal clearances of salicyluric acid were higher during the infusions than after, the differences were not statistically significant. There was no statistically significant correlation between the renal clearance of salicyluric acid (corrected for flow rate or uncorrected) and urine pH or flow rate.

Urinary recovery of acetylsalicylic acid

Acetylsalicylic acid was measurable in the first few urine samples from 10, 6, 4, 4 and 4 patients with forced alkaline diuresis, control group, forced diuresis, alkali alone and forced alkaline diuresis plus frusemide groups respectively. The urinary recovery varied from 2.77 mg in patient No. 5 to 613 mg in patient No. 17 both in the forced alkaline diuresis group (Table 4.15). The mean urinary recoveries of the above patients were 250, 125, 112, 77 and 42 mg with forced alkaline diuresis plus frusemide, alkali alone, forced alkaline diuresis, forced diuresis and control groups, respectively. There were no statistically significant differences between the groups.

Urinary recovery of salicylic acid

The urinary recovery of salicylic acid during the first 4 and /

TABLE 4.15.

URINARY RECOVERY OF ACETYLSALICYLIC ACID (mg)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	mg	No.	mg	No.	mg	No.	mg	No.	mg
1	89.0	2	7.03	53	21.2	27	10.2	37	71.5
4	8.61	5	2.77	54	535	29	97.4	44	54.8
6	40.7	10	40.0	55	373	31	76.0	47	133
7	5.72	14	37.4	58	71.7	33	124	49	241
13	65.8	17	613						
52	42.5	19	82.5						
		20	14.4						
		22	11.5						
		23	248						
		24	65.1						
Mean:	42.1		112		250		77		125
S.D.:	27.3		190		190		37.7		62.3

Analysis of variance : F - Ratio = 1.185 $p > 0.05$

and 16 hours after admission are shown in Tables 4.16 and 4.17. The mean urinary recoveries over the initial 4 hours (infusion period) were 2435, 2172, 1551, 437 and 162 mg with alkali alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis and control groups, respectively. There were no statistically significant differences between the alkalinisation groups, but there were significant differences between these and forced diuresis or control groups ($p < 0.02$ and $p < 0.001$ respectively). The difference between the salicylic acid urinary recovery of forced diuresis and control group was not statistically significant.

The mean urinary recovery during the first 16 hours was 3871, 3423, 3599, 1529 and 376 mg respectively. There were significant differences in the urinary recoveries of salicylic acid between the alkalinisation regimes (particularly alkali alone) and the forced diuresis or control groups ($p < 0.02$ and $p < 0.001$ respectively), but there were no statistically significant differences between the alkalinisation groups. However, the urinary recovery of salicylic acid (0-16 hours) was significantly higher in the forced diuresis than the control group ($p < 0.01$)

The mean urinary recoveries of salicylic acid in each group were normalised to an admission plasma concentration of 475 μg/ml and are given in Table 4.18. The values from 0-4 hours were 2634, 2186, 1482, 441 and 235 mg and from 0-16 hours 4188, 3454, 3439, 1541 and 544 mg with alkali alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis and control groups respectively.

Urinary /

TABLE 4.16.

URINARY RECOVERY OF SALICYLIC ACID 0-4 HOURS (mg)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	mg	No.	mg	No.	mg	No.	mg	No.	mg
1	332	2	2374	53	1639.5	27	296	37	1229.5
4	148	3	1052	54	3108	29	185	38	1406
6	174	5	1660	55	2344	30	52	44	2120
7	335.5	10	1934	57	621	31	1413.5	47	2230.5
8	N.A.*	14	977	58	3336	32	367	49	2044
9	171	15	820	59	1985	33	311	51	5581
13	533	17	1894						
16	16.5	19	2696						
34	88	20	1802						
35	44	21	3271						
36	139.5	22	818						
43	19	23	721						
45	105	24	308						
48	124	25	1426.5						
50	36	26	2543						
52	162	41	517						
Mean:	162		1551		2172.3		437.4		2435
S.D.:	± 141.2		866		998.5		491		1594

Analysis of variance: F - Ratio = 10.677 $p < 0.01$

* not available

TABLE 4.17.

URINARY RECOVERY OF SALICYLIC ACID 0-16 HOURS (mg)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	mg	No.	mg	No.	mg	No.	mg	No.	mg
1	1035	2	3078	53	2487	27	778	37	3480
4	169	3	1982	54	4925	29	816	38	2322
6	638	5	3586	55	3762	30	581	44	5359
7	558	10	5344	57	1664	31	3890	47	4532
8	N.A.*	14	N.A.	58	3855	32	2042	49	4980
9	297	15	N.A.	59	3901	33	1064	51	2552
13	1061	17	5031						
16	84	19	4807						
34	315	20	3583						
35	115.5	21	3471.5						
36	371	22	2759						
43	51	23	3646						
45	383.5	24	3420						
48	242	25	3914						
50	88	26	3212						
52	234	41	2546						
Mean:	376		3598.5		3432.3		1528.5		3871
S.D.:	± 320.5		943.0		1162.4		1267.0		1278.5

Analysis of variance: F - Ratio = 3.2643 $p < 0.05$

* not available

TABLE 4.18.

URINARY RECOVERY OF SALICYLIC ACID NORMALISED TO ADMISSION PLASMA CONCENTRATION OF 475 μg/ml

Group	Admission plasma salicylate (μg/ml)	Urinary recovery (mg)	
		0 - 4 hr	0 - 16 hr
Control	328	235	544
Forced alkaline diuresis (FAD)	497	1482	3439
FAD plus frusemide	472	2186	3454
Forced diuresis	471	441	1541
Alkali alone	439	2634	4188

Urinary recovery of salicyluric acid

The urinary recoveries of salicyluric acid over the first 4 and 16 hours are shown in Tables 4.19 and 4.20. Except in the control, less salicyluric acid was excreted than salicylic acid. The mean recoveries during the first 4 hours were 444, 427, 329, 244, 231 mg and from 0-16 hours after admission were 1499, 1119, 1243, 1116, 1117 mg with forced alkaline diuresis plus frusemide, forced alkaline diuresis, alkali alone, control group and forced diuresis respectively. There were no statistically significant differences between the salicyluric acid urinary recoveries in any of the groups.

The mean urinary recovery of acetylsalicylic, salicylic and salicyluric acids in each group for the first 4 and 16 hours is shown in Figure 4.9. The mean ratios of recovery of salicylic to salicyluric acid (expressed as salicylic acid equivalent) from 0-16 hours were 4.54, 4.40, 3.23, 1.93 and 0.48 with forced alkaline diuresis, alkali alone, forced alkaline diuresis plus frusemide, forced diuresis and control groups respectively. These ratios were much higher during the infusion period (0-4 hours) particularly with alkali alone (5.66, 10.5, 6.91, 2.68 and 0.94 respectively). The ratios of the recovery of salicylic to salicyluric acid for each patient during the whole hospitalisation period are given in Table 4.21. The mean ratio for the forced alkaline diuresis group was higher (but not significantly so) than the other alkalinisation groups. This was due to the short period of urine collections in 2 patients (Nos. 14 and 19). There was a signficant correlation between the ratio of urinary salicylic to salicyluric acids ratio and urine pH ($r = 0.47$, $p < 0.01$).

Urinary /

TABLE 4.19.

URINARY RECOVERY OF SALICYLURIC ACID 0-4 HOURS (mg)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	mg	No.	mg	No.	mg	No.	mg	No.	mg
1	255	2	312	53	343	27	516	37	389
4	262	3	156	54	482	29	66	38	298
6	140	5	402	55	434.5	30	172	44	315
7	390	10	515	57	576	31	298	47	372
8	N.A.*	14	171	58	642	32	117.5	49	348
9	158	15	366	59	185.5	33	214	51	249
13	350	17	438						
16	378	19	208.5						
34	133	20	62						
35	322	21	715						
36	185	22	261.5						
43	343	23	61						
45	101.5	24	456						
48	173	25	607						
50	249	26	722						
52	422	41	1372						
Mean:	243.5		427		444		230.6		328.5
S.D.:	± 100.2		327		164.4		161		51.7

Analysis of variance : F - Ratio = 1.942 $p > 0.05$

* not available

TABLE 4.20.

URINARY RECOVERY OF SALICYLURIC ACID 0-16 HOURS (mg)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	mg	No.	mg	No.	mg	No.	mg	No.	mg
1	1126	2	789.5	53	1272	27	1698	37	1321
4	544	3	686	54	1447	29	559	38	974
6	1482	5	1400	55	1607.5	30	770	44	1324
7	918	10	1738	57	2260	31	1364	47	1493
8	N.A.*	14	N.A.	58	1341	32	1056	49	1389
9	532	15	N.A.	59	1066	33	1256	51	956
13	979	17	1062						
16	2593	19	474						
34	750	20	285						
35	1423	21	747						
36	767	22	896						
43	1594	23	335						
45	384	24	1741						
48	1370	25	1359						
50	1152	26	1479						
52	1125	41	2672						
Mean:	1116 ±		1119		1499		1117.2		1243
S.D.:	549		660		414.2		413		224
Analysis of variance:		F - Ratio = 0.684 p > 0.05							

* not available

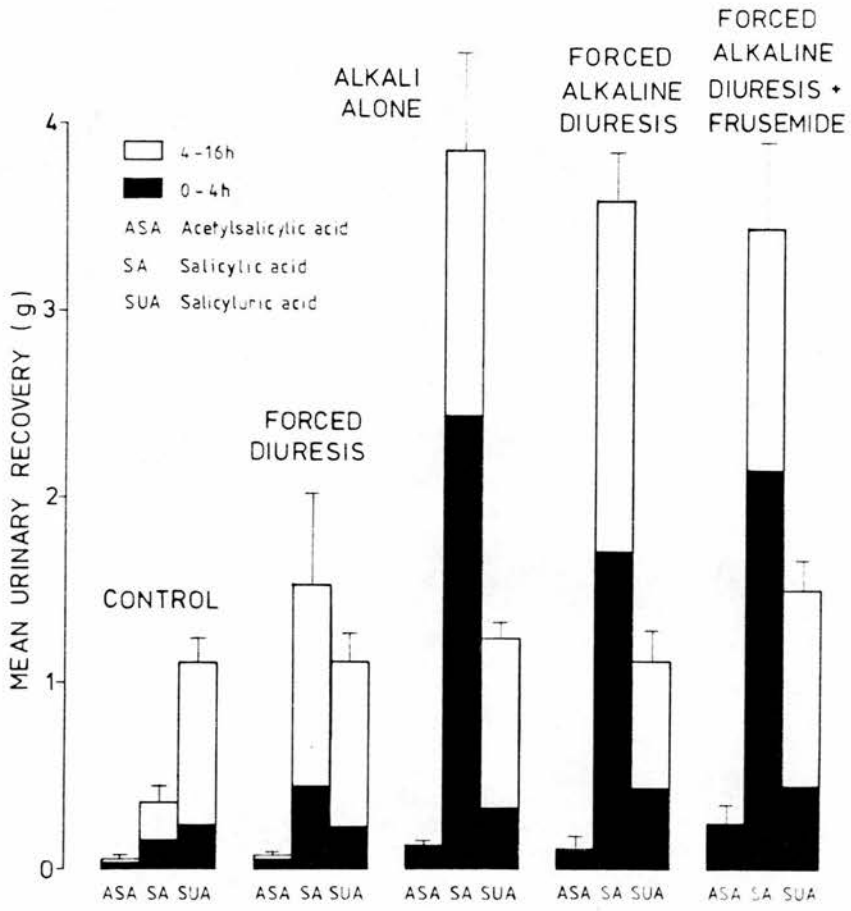

Figure 4.9. Mean urinary recovery of acetylsalicylic, salicylic and salicyluric acids over the first 4 and 16 hours after admission in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

TABLE 4.21.

THE RATIO OF URINARY RECOVERY OF SALICYLIC ACID TO SALICYLURIC ACID (0.708)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio	No.	Ratio
1	0.74	2	8.56	53	2.44	27	0.52	37	3.33
4	0.44	3	3.67	54	2.03	29	1.43	38	2.82
6	0.61	5	1.82	55	3.27	30	0.95	44	3.41
7	0.78	10	4.01	57	0.65	31	1.81	47	2.08
8	N.A.*	14	15.0	58	1.65	32	1.26	49	4.61
9	0.58	15	5.9	59	3.45	33	0.64	51	3.67
13	1.53	17	4.87						
16	0.14	19	14.1						
34	0.54	20	14.2						
35	0.10	21	6.56						
36	0.42	22	3.13						
43	0.14	23	15.3						
45	0.44	24	0.93						
48	0.27	25	2.71						
50	0.09	26	2.84						
52	0.15	41	1.08						
Mean:	0.46		6.54		2.41		1.10		3.32
S.D.:	± 0.38		5.22		1.08		0.49		0.85

Analysis of variance : F - Ratio = 9.0784 $p < 0.01$

* not available

Urinary excretion of glucuronide conjugates

Glucuronide conjugates of salicylic acid in the urine varied from 0 in a patient treated with alkali alone (No. 49) to 77% in a control (No. 52). The total urinary recovery of salicylic acid and percentage excreted as glucuronide conjugates are given in Table 4. 22. The mean urinary recoveries of total (free and conjugated) salicylic acid were 4523, 4330, 2768 and 966 mg with the alkali alone, forced alkaline diuresis, forced diuresis and control groups respectively. The mean percentages which were conjugated were in reverse, 60, 42, 15 and 13 per cent in the control, forced diuresis, forced alkaline diuresis and alkali alone groups respectively. There were significant differences in the urinary recoveries of total salicylic acid between the alkalinisation regimes and controls ($p < 0.001$), but not between the alkalinisation regimes and forced diuresis or between the forced alkaline diuresis and alkali alone regimes. The percentage excreted as conjugates was significantly higher in the control and forced diuresis groups than in the forced alkaline diuresis or alkali alone groups ($p < 0.01$) and ($p < 0.005$).

The urinary excretion of total and glucuronide conjugates of salicyluric acid is shown in Table 4.23. The urinary recovery of total salicyluric acid was lower than that of the salicylic acid in all groups except for the control group in which it was more than double the urinary recovery of salicylic acid. There were no statistically significant differences in the urinary recoveries of total salicyluric acid between the groups. There were significant differences in the percentage excreted as glucuronide conjugates between the forced alkaline diuresis and forced diuresis or alkali alone groups, but not the control group ($p < 0.05$).

Correlation /

TABLE 4.22. TOTAL URINARY RECOVERY OF SALICYLIC ACID AND PERCENTAGE CONJUGATED WITH GLUCURONIDES

Control			Forced diuresis			Alkali alone			Forced alkaline diuresis		
No.	Total (mg)	%	No.	Total (mg)	%	No.	Total (mg)	%	No.	Total (mg)	%
34	698	46.4	27	3176	56.5	37	4976	9.87	20	4312	9.88
35	709	82.6	29	1368	36.5	38	3048	12.1	22	3365	10.3
45	1608	58.6	30	1032	45.5	44	5064	21.4	24	4986	22.0
48	963	68.6	31	5199	24.9	47	6397	22.5	25	3879	0.52
50	559	24.3	32	3495	40.3	49	4871	0	26	3905	11.0
52	1258	77.3	33	2336	48.5	51	2782	11.3	41	5534	37.2
Mean:	966	59.6		2768	42		4523	12.9		4330	15.2
	±										
S.D.:	400.2	21.7		1534	10.9		1367	8.3		799.4	12.8

Analysis of variance: Total: F-Ratio = 13.06 $p < 0.01$

% : F-Ratio = 14.79 $p < 0.01$

TABLE 4.23. TOTAL URINARY RECOVERY OF SALICYLURIC ACID AND PERCENTAGE CONJUGATED WITH GLUCURONIDES

Control			Forced diuresis			Alkali alone			Forced alkaline diuresis		
No.	Total (mg)	%	No.	Total (mg)	%	No.	Total (mg)	%	No.	Total (mg)	%
34	1056	9.56	27	4140	9.08	37	2071	10.8	20	442	0
35	2086	29.7	29	975	10.4	38	1796	12.5	22	1652	6.80
45	2365	15.4	30	1111	24.4	44	2551	18.3	24	5848	2.80
48	2373	12.7	31	3858	14.1	47	3736	14.6	25	1824	1.81
50	1665	12.4	32	3016	15.1	49	1609	14.1	26	1796	2.00
52	2761	6.16	33	3134	17.8	51	1015	13.5	41	3575	20.4
Mean:	2051	14.3		2706	15.2		2130	14.0		2523	5.6
S.D.	608	8.2		1357	5.5		937	2.5		1912	7.6

Analysis of variance

Total F-Ratio = 0.35 $p > 0.05$

% F-Ratio = 2.95 $p < 0.05$

Correlation between renal clearance of salicylic acid and urine pH or flow rate

There were significant positive correlations between the renal clearances of salicylic acid and urine pH in each group ($p < 0.001$) with correlation coefficients ranging from 0.48 with forced alkaline diuresis plus frusemide to 0.68 with alkali alone. These correlations were more significant (except for forced alkaline diuresis with frusemide) when the salicylic acid clearance was corrected for flow rate (equivalent to the ratio of urine to plasma concentration).

The correlation and regression coefficients of the ratios of urine to plasma concentrations of salicylic acid against urine pH and flow rate are shown in Table 4.24. The positive correlation between renal clearance of salicylic acid corrected for flow rate and urine pH was also significant for forced alkaline diuresis with frusemide.

The renal clearance of salicylic acid corrected for flow rate for the 39 urine samples from the 6 patients treated with alkali alone and 39 representative samples from 13 of the control patients (first, middle and last urine sample from each) were plotted against the corresponding urine pH to demonstrate the highly significant relationship between the clearance and pH without the effects of diuresis (Fig.4.10). There were significant negative correlations between the ratios of urine to plasma salicylic acid concentrations and urine flow rate in the forced alkaline diuresis and controls ($r = -0.43$, $p < 0.001$ and $r = -0.24$, $p < 0.05$ respectively), but not in the alkali alone and forced alkaline diuresis with frusemide. There was a significant positive correlation between the ratio of urine to plasma concentrations of salicylic acid and urine flow rate in /

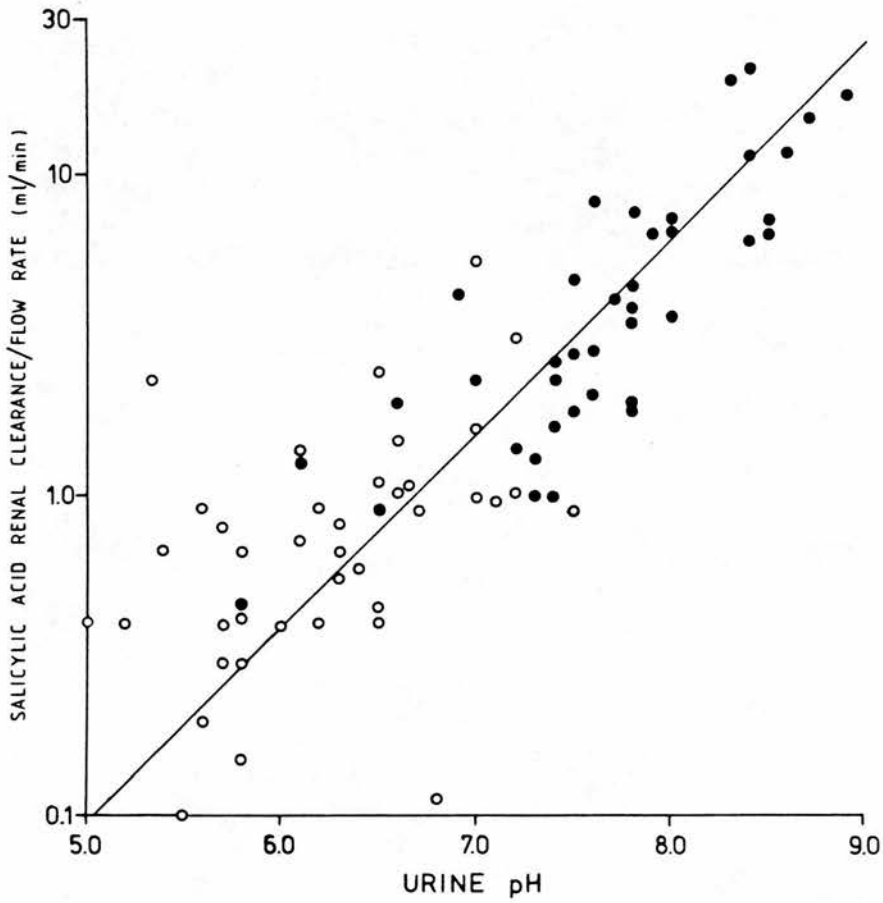

Figure 4.10. Correlation between the renal clearance of salicylic acid and urine pH in 6 patients with aspirin poisoning receiving alkali alone (●) and 13 control patients (o).

in the forced diuresis group. There were positive correlations between the urine flow rate and pH in all groups except in the alkali alone ranging from $p < 0.05$ with the control to $p < 0.001$ with forced fiuresis which was consistent with the significant positive correlation between the ratio of urine to plasma salicylic acid concentration and urine flow rate.

Multiple regression analysis

Multiple correlations between the ratio of urine to plasma salicylic acid concentrations and urine pH and flow rate were significant in all groups ($p < 0.01$) (Table 4.24.). The multiple correlation coefficients between the ratio of urine to plasma salicylic acid concentrations and urine pH and flow rate were 0.85, 0.77, 0.69, 0.68 and 0.51 with alkali alone, forced diuresis, control, forced alkaline diuresis and forced alkaline diuresis with frusemide respectively. The regression coefficients for pH were 6.26, 2.06, 0.531, 0.491 and 0.318 with alkali alone, forced alkaline diuresis, control, forced diuresis and forced alkaline diuresis with frusemide, respectively. The regression coefficients for flow rate were all negative.

The multiple regression equation for the alkali alone regime was :

$$r = 6.26\ pH - 0.415F - 0.33$$

(r is the ratio of urine to plasma salicylic acid concentrations, pH is the urine pH and F is the urine flow rate). Thus for each increase of one unit in urine pH the ratio will increase 6.26 fold, whereas for the flow rate there is slight decrease. The equation for the forced alkaline diuresis regime (second most effective) is :

$$r = 2.06\ pH - 0.136F - 0.24$$

and /

TABLE 4.24. COMPARISON OF CORRELATIONS BETWEEN THE RATIO OF URINE TO PLASMA SALICYLIC ACID CONCENTRATIONS AND URINE pH AND FLOW RATE

Group	Correlation coefficient				Multiple regression				Multiple correlation coefficient	
					Urine pH		Flow rate			
	Ratio vs urine pH	Ratio vs urine flow	Urine pH vs flow rate	Degrees of freedom	Regression coefficient	Standard error	Regression coefficient	Standard error	Ratio vs urine pH & flow rate	Degrees of freedom
Control	0.49***	-0.24*	0.27*	85	0.531	0.18	-0.102	0.04	0.69**	16 & 67
Forced diuresis	0.57***	0.30*	0.61***	66	0.491	0.06	-0.018	0.01	0.77**	16 & 99
Alkali alone	0.70***	-0.25	0.22	37	6.26	0.88	-0.415	0.11	0.85**	7 & 31
Forced alkaline diuresis	0.32***	-0.43***	0.20*	115	2.06	0.55	-0.136	0.02	0.68**	16 & 99
Forced alkaline diuresis with frusemide	0.33*	-0.24	0.40**	51	0.318	0.28	-0.02	0.01	0.51**	7 & 57

* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

and for the control, forced diuresis and forced alkaline diuresis with frusemide equations are as follows :

$$r = 0.531\ pH - 0.102F - 0.108$$

$$r = 0.491\ pH - 0.018F - 0.102$$

$$r = 0.318\ pH - 0.02F - 0.22$$

Therefore increasing the pH without diuresis enhanced the ratio of urine to plasma salicylic acid concentrations. The least effect of urine pH was shown in the forced alkaline diuresis with frusemide treatment.

The correlation between the renal clearance of unbound salicylic acid and urine pH (using individual urine samples) was also significant ($r = 0.55$, $p < 0.001$).

(g) Discussion

Treatment regimes

Since the urine pH was significantly higher with the alkalinisation regimes than in the other groups, and urine flow rate was greater in the forced diuresis and forced alkaline diuresis groups and enhanced by frusemide, all treatment regimes effectively increased the urine pH or flow rate. The alkaline urine pH in the forced diuresis regime might reflect a greater degree of hyperventilation in the patients who had higher plasma salicylate concentrations than the controls. Although the amount of sodium bicarbonate was the same in the alkali alone and forced alkaline diuresis regimes, the urine pH was lower in the latter, possibly due to acid dextrose infusion, and the diluting effect of diuresis.

Lawson et al. (1969) reported a mean urinary pH below 7.0 with similar /

similar forced cocktail diuresis, and this could be due to oxidation of laevulose in alkaline solution. The difference between the forced cocktail diuresis and forced alkaline diuresis regime used here, is that glucose replaced laevulose and sodium bicarbonate was added just before use rather than using the mixture already made with sodium bicarbonate in the Pharmacy Department.

Plasma half-life of salicylic acid

The plasma half-lives of salicylic acid were all much shorter during the infusion than after. This must have been largely due to haemodilution (Chapter 1, Section V) and an increased volume of distribution, since the urinary recovery of salicylate over that period was not nearly sufficient to account for the fall observed. The apparent plasma half-lives during the infusion were significantly lower with the alkalinisation regimes than with the forced diuresis. Thus alkalinisation also shortens the plasma half-life of salicylic acid by enhancing the renal excretion. The plasma half-life of salicylic acid during infusion is, therefore, not a valid index of removal of salicylic acid from the body and its use as such is misleading. Although there were no statistically significant differences in the apparent plasma half-lives between the alkalinisation groups, the rate of fall of salicylic acid concentration during the infusion was significantly greater with forced diuresis than with alkali alone.

The plasma concentrations of salicylic acid after infusion (4-16 hours) decreased with different slopes for each treatment.

The /

The plasma half-life of salicylic acid over this period was compatible with the salicylate elimination. The plasma concentrations of salicylic acid after infusion declined with similar initial slope in 3 of the patients treated by alkali alone. In one of them (No. 49) the plasma salicylic acid half-life was even shorter after the infusion (2.62 hr). This patient had the highest urine pH and a total of more than 5 g of salicylic acid was recovered in the urine. There is no report of such a short plasma half-life of salicylic acid with overdosage in the literature. In fact, the serial plasma salicylate concentrations and half-lives reported were measured by non-specific analytical methods (Done, 1960; Beveridge et al., 1964; Cumming et al., 1964; Morgan and Polak, 1969; Lawson et al., 1969; Ferguson and Boultros, 1970; James and Martinak, 1975; Temple et al., 1976). However, the reported plasma salicylate half-lives with different methods of intravenous treatment ranged from 5 hours in a patient who was treated by forced alkaline diuresis to over 24 hours in two patients who received forced cocktail diuresis (Lawson et al., 1969).

The shortest mean plasma half-life of salicylic acid in the alkali alone and the longest in the forced diuresis group were consistent with the renal clearances and urinary recovery. The alkali alone regime, therefore, was the best for the removal of salicylic acid from the body.

As expected, glycine conjugation of salicylic acid was saturated very early (usually before admission) and the plasma salicyluric acid concentrations remained very low in all patients. The fall in the plasma salicyluric concentrations during the infusions might also be due to haemodilution.

Total /

Total body clearance and volume of distribution of salicylic acid

The total body clearance was higher during than after the infusion in all treated groups which is consistent with the higher renal clearance and urinary recovery of salicylic acid. The apparent volumes of distribution of salicylic acid did not change significantly after the infusion. The concentration-dependent volume of distribution of total salicylic acid reported by Levy and Yaffe (1974) was confirmed by using the individual samples in 5 patients. In addition, it was confirmed that this reflects protein binding and in fact, the volume of distribution of unbound salicylic acid declined with increasing concentration.

Renal clearance, urine pH and flow rate

The increased renal clearance of salicylic acid with forced diuresis was probably due to changes in both urine pH and flow rate since they were higher in the treated groups than in the control group, particularly during the infusion period.

The high renal clearances of acetylsalicylic acid, unbound salicylic acid and salicyluric acid (> 100 ml/min) indicate their active tubular secretion which is consistent with previous reports (Schacter and Manis, 1958; Bedford, Cumming and Martin, 1965). The urine pH did not affect the urinary excretion and renal clearance of salicyluric acid presumably because it is not reabsorbed.

The higher renal clearance of salicylic acid in the alkalinisation groups compared with forced diuresis and controls is due to the higher urine pH and decreased renal tubular reabsorption. The correlation between the renal clearance of salicylic acid corrected for flow rate and urine pH showed the highest correlation coefficient /

coefficient with the alkali alone regime (Table 4.24). The multiple regression analysis between the ratio of urine to plasma salicylic acid concentration and urine pH and flow rate also gave the highest multiple correlation coefficient for the alkali alone group and the least for forced alkaline diuresis with frusemide. These findings were compatible with the observed renal clearance and excretion of salicylic acid. The lack of increased efficacy of forced alkaline diuresis with frusemide is consistent with the findings of Berg (1977b).

The higher renal clearance of unbound salicylic acid and its good correlation with urine pH is very similar to the results of Smith et al. (1946).

Urinary recovery

The urinary recovery of acetylsalicylic acid accounted for less than 0.5% of the total urinary recovery of salicylates which is due to its rapid hydrolysis to salicylic acid. The proportional urinary recovery of salicylic acid increased significantly and salicyluric acid decreased with a higher pH, and the ratio of salicylic acid to salicyluric acid was pH-dependent. There is no such finding in the literature since acetylsalicylic and salicyluric acids have not been measured separately following overdosage.

Although the urinary recovery of total salicylic acid (including the glucuronide conjugates) was higher with the alkalinisation regimes, the percentage conjugated with glucuronide was less /

less than in the forced diuresis or control groups. This might be due to excretion of more unchanged salicylic acid because of the effect of pH on its renal clearance. Since the highest renal clearance and urinary recovery of salicylic acid was achieved with alkali alone, it was more effective than the other regimes, including forced alkaline diuresis.

(h) Summary and conclusions

The effects of changes in urine pH and flow rate on the distribution and elimination of acetylsalicylic acid and its metabolites were studied in 34 adult patients with moderate to severe aspirin poisoning (plasma salicylate 400 - 800 µg/ml).

The patients were treated with one of four intravenous regimes of fluid and alkali over 3 hours according to the admission plasma salicylate concentrations measured by a ward side-room assay (modified Trinder's method). Sixteen patients received forced alkaline diuresis (6 L isotonic dextrose/saline solution plus 18.9 g sodium bicarbonate plus 9 g potassium chloride) 6 forced diuresis (6 L isotonic dextrose/saline plus 9 g potassium chloride), 6 alkali alone (18.9 g sodium bicarbonate and 4.5 g potassium chloride in 1½ L) and 6 forced alkaline diuresis with 80 mg frusemide given intravenously. No specific treatment was given to 16 control patients with mild aspirin overdosage.

Acetylsalic ylic acid was rapidly hydrolysed to salicylic acid and was only detected when absorption was delayed (25 patients).

All /

All treatments produced similar initial rapid falls in plasma salicylic acid concentrations during the infusion period (0-4 hours), but urinary salicylate excretion was only increased with the alkanin-isation regimes. Haemodilution and an increased volume of salicylic acid distribution probably contributed to this initial fall, par-ticularly in the non-alkalinisation regime.

From 4-16 hours the mean apparent plasma half-lives were 30, 31, 12, 14 and 9 hours in the control group, forced diuresis, forced alkaline diuresis, forced alkaline diuresis with frusemide and alkali alone regimes respectively.

Total body clearance, renal clearance and urinary recovery of salicylic acid were significantly higher with the alkalinisation regimes than with the forced diuresis alone.

The renal clearance of salicylic acid was a function of both urine pH and flow rate in all groups. There was a direct relation-ship with the latter but the influence of pH increased dramatically above 7.0 where it completely dominated the elimination kinetics of salicylic acid.

The mean renal clearances of salicylic acid during the first 16 hours after admission were 1.4, 4.4, 18, 13 and 24 ml/min with the control, forced diuresis, forced alkaline diuresis, forced alkaline diuresis plus frusemide and alkali alone groups, respectively.

The urinary recoveries of salicylic acid from 0-16 hours after admission were 3871, 3599, 3434, 1529 and 376 mg with alkali alone, forced /

forced alkaline diuresis, forced alkaline diuresis plus frusemide, forced diuresis and control groups, respectively. The ratio of salicylic to salicyluric acid urinary recovery was highly pH-dependent.

The plasma concentrations of salicyluric acid were uniformly low (< 10 μg/ml) throughout the study in all groups reflecting saturation of glycine conjugation of salicylic acid. The very high renal clearance of salicyluric acid was independent of urine pH and flow rate.

Administration of alkali alone was more effective than the other regimes including the forced alkaline diuresis, for the removal of salicylic acid in acetylsalicylic acid poisoning.

SECTION V

EFFECTS OF PROSTAGLANDIN-MEDIATED CHANGES IN FLUID AND ELECTROLYTE BALANCE AND SALICYLATE DISTRIBUTION FOLLOWING OVERDOSAGE

SECTION V

Chapter 1.

THE EFFECTS OF TREATMENT WITH FLUID AND ALKALI ON FLUID RETENTION IN ASPIRIN POISONING

(a) Introduction

It is well known that acetylsalicylic acid and some of its metabolites inhibit the biosynthesis of prostaglandins (Vane, 1971; Smith and Willis, 1971; Flower, 1974; Shen, 1979, Smith et al., 1979; Flower et al., 1980). Aspirin exerts two separate effects on prostaglandin formation in vivo, a rapid action of the intact molecule on easily accessible tissue and a later action due to its hydrolysis to salicylic acid (Smith et al., 1979).

In acetylsalicylic acid poisoning, sodium and water retention is likely to occur (Berg, 1977b), but because of insensible loss of fluid due to vomiting, sweating and hyperventilation, dehydration may be a problem in patients who were admitted a long time after ingestion. However, a positive fluid balance, as calculated from fluid intake and output was reported as a fluid "deficit" (Lawson et al., 1969) and fluid retention has been reported in patients with aspirin overdosage treated by forced diuresis (Savage et al., 1969; Temple et al., 1976). In the study by Lawson et al. (1969), fluid "deficit" varied from 0.6 to 3.6 litres in patients treated with oral fluids only compared with 1.8 to 11.1 litres in patients treated by forced diuresis. In the case report by Savage et al.(1969), there was retention of 3.7 litres of fluid following treatment with 190 g mannitol. Temple et al. (1976) reported positive fluid balances of 417 ml /

417 ml and 1150 ml in a 4 month old male and a 21 month old female respectively, but weight changes of the patients were not given.

Since insensible loss in aspirin poisoning may be considerable, fluid balance without serial measurement of the patient's weight is of limited use. Serial measurements of haematocrit or plasma proteins could be used as an index of haemodilution following forced diuresis, but have only been reported by Lawson et al. (1969).

This study was undertaken to establish the changes in fluid balance, patient's weight and haemoconcentration in aspirin poisoning with and without treatment by diuresis.

(b) Patients and methods

Thirty-four patients aged 16-55 years of age (17 males and 17 females) with mild to severe aspirin poisoning were studied. Eight patients (4 males and 4 females) received no specific treatment (control patients, Chapter 1, Section IV), 8 (4 males and 4 females) were treated with forced alkaline diuresis, 6 (3 males and 3 females) with forced diuresis, 6 (3 males and 3 females) with alkali alone and 6 (3 males and 3 females) with forced alkaline diuresis plus frusemide (Chapter 2, Section IV). The patients were weighed before and after gastric lavage, at the end of fluid infusion (at 4 hours after admission for the controls) and then 8 hourly until discharge. Fluid intake (i.v. and oral) and output (urine, vomit and other) measurements were recorded.

Blood samples were taken for determination of the haematocrit at the same time as for drug measurement (see Section IV) and 3 further samples were taken (in the Lithium Heparin tubes) for biochemical investigations on admission, at the end of infusion (at 4 hours after admission /

admission for the controls) and at 16 hours after admission. The plasma albumin concentration was measured by the Technicon Sequential Multiple Analysis plus Computer (SMAC) System in the University Department of Clinical Chemistry, Royal Infirmary of Edinburgh.

Comparisons between the groups were performed using one way analysis of variance. If the differences were significant ($p < 0.05$) the independent two-tailed Student t-test was used for the comparison of each group. For the comparison of values before and after infusion (4 and 16 hours after admission) the paired two-tailed Student t-test was used.

(c) Results

Fluid balance and weight change

The weights of the patients did not change significantly after gastric lavage. The overall mean change was +50.4 ± 360 (S.D.) g.

The fluid balance and weight changes for each patient at 4 hours after admission are summarised in Tables 5.1. and 5.2. The fluid balances were all positive except for one control patient (No. 35) and 2 (Nos. 44 and 49) with alkali alone treatment. The mean of fluid balance increases were 4621, 3565, 1577, 434 and 219 ml with forced diuresis, forced alkaline diuresis, forced alkaline diuresis plus frusemide, alkali alone and control groups respectively. The weight changes at 4 hours were consistent with the fluid balance and the mean differences for each group were +3833, +2513. +326, -417 and -143 g respectively. There were highly significant differences in the fluid balance and weight gain between the forced alkaline diuresis group and the alkali alone or control groups ($p < 0.001$). There were also significant differences between the forced alkaline diuresis and forced /

TABLE 5.1.

FLUID BALANCE* AT 4 HOURS AFTER ADMISSION (ml)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml	No.	ml	No.	ml	No.	ml	No.	ml
1	0	3	+4000	53	+3350	27	+5400	37	+750
34	+550	14	+4000	54	+1230	29	+2325	38	+130
35	-950	20	+3000	55	+ 500	30	+4600	44	-475
36	+400	22	+2800	57	+1150	31	+4800	47	+1100
45	+800	24	+4220	58	+1655	32	+5400	49	-350
48	0	25	+3700	59	+1575	33	+5200	51	+1450
50	+450	26	+4100						
52	+500	41	+3700						
Mean:	+219		+3565		1577		4621		434
S.D.:	544		556		960		1171		789

Analysis of variance: F-Ratio = 39.25 $p < 0.01$

* Fluid intake - fluid output

TABLE 5.2.

WEIGHT CHANGE AT 4 HOURS FOLLOWING ADMISSION (g)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	g	No.	g	No.	g	No.	g	No.	g
1	N.A.*	3	+2000	53	-500	27	+4100	37	+700
34	-500	14	+2000	54	-900	29	+2000	38	-1400
35	-500	20	+2500	55	+900	30	+4500	44	-500
36	-500	22	+2600	57	+1200	31	+4400	47	+500
45	+1200	24	+3500	58	+800	32	+3000	49	-300
48	-250	25	+3500	59	+455	33	+5000	51	-500
50	+150	26	+3500						
52	-600	41	+500						
Mean:	-143		+2513		+326		+3833		-417
S.D.:	644		1036		839		1118		671

Analysis of variance : F-Ratio = 26.207 $p < 0.01$

* not available

forced diuresis ($p < 0.05$) and forced alkaline diuresis with frusemide groups ($p < 0.005$), but not between the forced alkaline diuresis with frusemide and alkali alone or the control groups.

The positive fluid balances decreased with time in the forced diuresis and forced alkaline diuresis groups, and increased in the other 3 groups. The patients' weights also decreased in the forced alkaline diuresis with frusemide group. Thus the differences were less marked at 28 hours after admission. The fluid intake up to 28 hours varied from 1850 ml in a control patient (No. 50) to 10,775 ml in a patient (No. 27) treated with forced diuresis. The fluid balance and weight changes at 28 hours after admission are shown in Tables 5.3. and 5.4. The mean positive fluid balances were 3229, 3092, 2106, 1111 and 1151 ml with the forced alkaline diuresis, forced diuresis, forced alkaline diuresis plus frusemide, alkali alone and control groups respectively, while the corresponding mean weight changes were 1393, 1550, 212, 190 and 267 g. There were significant differences between the fluid balance with forced diuresis or forced alkaline diuresis and alkali alone or control ($p < 0.001$) and forced alkaline diuresis with frusemide groups ($p < 0.01$). There were no statistically significant differences between the forced diuresis and forced alkaline diuresis, nor between the other 3 groups. Similar, but less significant differences in weight gain were found between the groups. The differences in fluid balance and weight gain were less at 28 hours than at the 4 hours in all groups.

Changes in haematocrit and plasma albumin

The mean serial haematocrit for each group of patients plotted against the time after admission is shown in Figure 5.1. and the

percent /

TABLE 5.3.

FLUID BALANCE* AT 28 HOURS AFTER ADMISSION (ml)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml	No.	ml	No.	ml	No.	ml	No.	ml
1	+705	3	+3025	53	+2435	27	+3625	37	+125
34	N.A.**	14	N.A.	54	+2824	29	+1575	38	+450
35	N.A.	20	+3225	55	N.A.	30	N.A.	44	+1325
36	+900	22	+2700	57	+918	31	+4310	47	+2845
45	+1438	24	+4705	58	+2930	32	+3450	49	+810
48	+1075	25	+2630	59	+1425	33	+2500	51	N.A.
50	+590	26	+3230						
52	+2200	41	+3090						
Mean:	+1151		+3229		+2106		+3092		+1111
S.D.:	594		693		891		1066		1067

Analysis of variance : F-Ratio = 8.14 $p < 0.01$

* Fluid intake - fluid output. ** not available.

TABLE 5.4.

WEIGHT CHANGE AT 28 HOURS FOLLOWING ADMISSION (g)

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	g	No.	g	No.	g	No.	g	No.	g
1	+200	3	+1000	53	-1000	27	+1500	37	-200
34	N.A.*	14	N.A.	54	+1100	29	+550	38	-1700
35	N.A.	20	+1500	55	N.A.	30	N.A.	44	+750
36	-800	22	+1500	57	+500	31	+1300	47	+2200
45	+2000	24	+3800	58	+460	32	+1400	49	-100
48	-500	25	+500	59	0	33	+3000	51	N.A.
50	+1000	26	+700						
52	-300	41	+750						
Mean:	+267		+1393		+212		+1550		+190
S.D.:	1058		1130		782		893		1428

Analysis of variance : F-Ratio = 2.234 $p > 0.05$

* not available

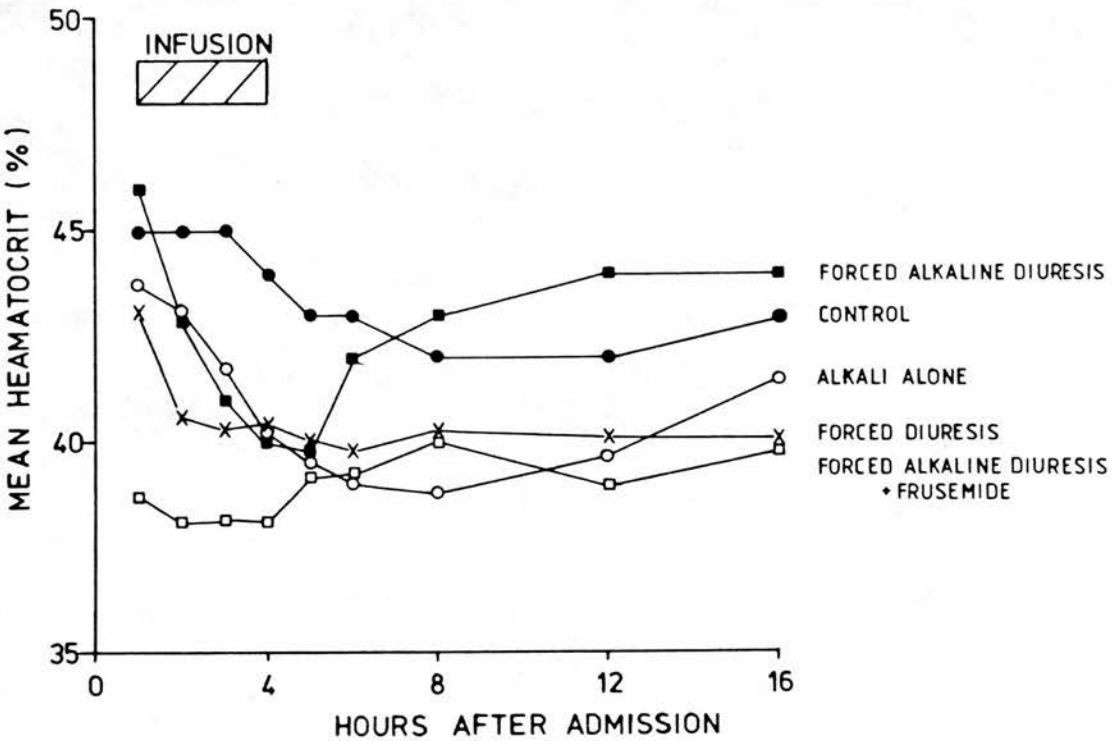

Figure 5.1. Changes in mean haematocrit in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

percent change for each patient is given in Table 5.5. The haematocrit increased after infusion, but the mean values were still lower at 16 hours than before the infusion (except for the forced alkaline diuresis with frusemide group). The maximum fall in the haematocrit was found in the forced alkaline diuresis group (12.9%) which was significantly higher than in the control ($p < 0.005$) or forced alkaline diuresis plus frusemide groups ($p < 0.001$). There were no statistically significant differences between the forced alkaline diuresis and forced diuresis or alkali alone groups.

The mean plasma albumin concentrations in each group before and after infusion and the following day are shown in Figure 5.2. and the individual percentage changes during the infusion are given in Table 5.6. The maximum fall in plasma albumin concentrations during the infusion was found with the forced alkaline diuresis group (20%) which corresponds with the fall in haematocrit. The fall in plasma albumin concentration was significant during the infusion in all groups ($p < 0.01$ to $p < 0.001$) except for forced alkaline diuresis with frusemide which again agrees with the haematocrit changes. The decline in plasma albumin was significantly greater with forced diuresis than with alkali alone ($p < 0.005$). The mean values increased after infusion with the forced diuresis and forced alkaline diuresis, but decreased in the other 3 groups (Fig. 5.2.)

(d) Discussion

Although fluid retention was considered in some reports (Savage et al., 1969; Temple et al., 1976) the weight changes of the patients were /

TABLE 5.5.

PERCENTAGE CHANGE IN HAEMATOCRIT DURING INFUSION

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	%	No.	%	No.	%	No.	%	No.	%
1	- 4.3	3	- 20	53	0	27	- 6.3	37	- 8.9
34	+ 2.5	14	- 16	54	- 10	29	- 11	38	0
35	0	20	- 9.1	55	- 8.3	30	- 9.1	44	- 2.4
36	+ 2.5	22	- 9.1	57	- 3.3	31	- 2.3	47	- 4.2
45	- 6.5	24	- 10	58	+ 2.3	32	- 7.5	49	- 15
48	0	25	- 6.3	59	+ 9.5	33	0	51	- 18
50	0	26	- 11						
52	- 5.8	41	- 22						
Mean:	- 1.45		- 12.9		- 1.63		- 6.03		- 8.08
S.D.:	3.59		5.70		7.20		4.16		7.20

Analysis of variance: F-Ratio = 7.237 $p < 0.01$

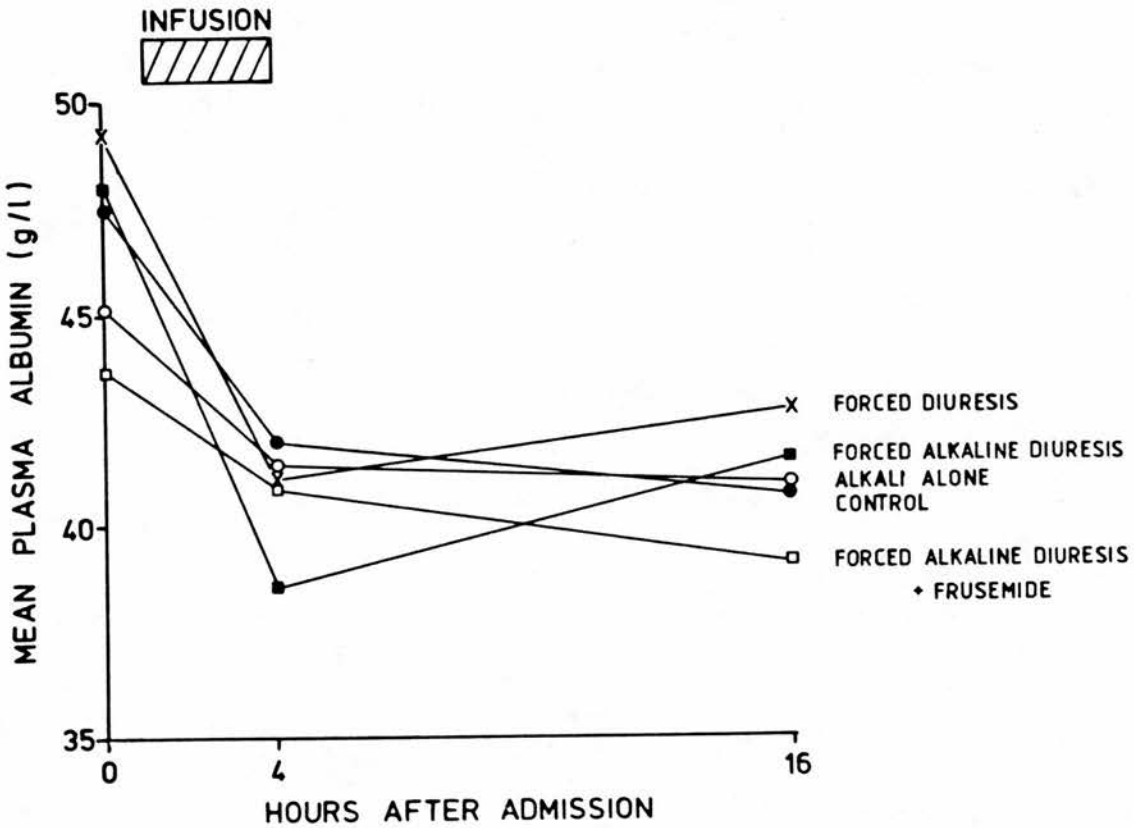

Figure 5.2. Changes in mean plasma albumin concentrations in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali (normal range 36 - 47 g/l).

TABLE 5.6.

PERCENTAGE CHANGE IN PLASMA ALBUMIN CONCENTRATION DURING INFUSION

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	%	No.	%	No.	%	No.	%	No.	%
1	- 15	3	-29	53	- 4	27	- 13	37	-6.7
34	- 11	14	-12	54	-7.5	29	- 16	38	- 8
35	- 8	20	-24	55	-20	30	- 22	44	-4.4
36	- 11	22	-13	57	+4.9	31	- 17	47	- 10
45	- 18	24	-19	58	-15	32	- 21	49	- 13
48	-4.5	25	-17	59	+4.9	33	-9.1	51	- 11
50	-4.5	26	-15						
52	- 14	41	-30						
Mean:	-10.8		-20		-6.1		-16.4		-8.9
S.D.:	4.9		7.0		10.2		4.9		3.1

Analysis of variance : F - Ratio = 5.32 $p < 0.01$

were only mentioned in one (Morgan et al., 1968). Since insensible loss occurs in aspirin poisoning due to sweating and hyperventilation, a positive fluid balance could not be interpreted as fluid retention unless the insensible loss was taken into account by measuring the changes in the patient's weight as well as fluid balance. Although the fluid balance at 4 hours was positive for all groups, the mean changes in weight were negative for the control and alkali alone groups at that time. The greater weight gains and fluid balance at the end of infusion in the forced diuresis and forced alkaline diuresis groups than in the other groups, are due to the higher infusion load (6 litres) and fluid retention. Frusemide induced a rapid diuresis in all 6 patients and thus the fluid balance and weight gain were significantly lower with this treatment than with forced diuresis or forced alkaline diuresis. This is consistent with the findings of Berg (1977b). As time passed, diuresis continued in the patients given forced diuresis and forced alkaline diuresis and more fluid was retained in the patients in the other groups. Therefore, the differences became less significant. The weight gains, however, were still significantly higher 28 hours after admission in the forced diuresis and forced alkaline diuresis than in the other groups.

Haemodilution during the infusion of 6 litres of fluid (forced diuresis and forced alkaline diuresis) and haemoconcentration with forced alkaline diuresis plus frusemide after infusion were confirmed by serial measurement of haematocrit and plasma albumin concentrations. Although the changes in haematocrit were similar in the forced diuresis and alkali alone groups, the fall in plasma albumin was significantly higher with forced diuresis than with alkali alone. In fact, the fall in the haematocrit curve with alkali alone was similar to that in /

in the control group (Fig. 5.1.). The increased mean haematocrit with forced alkaline diuresis plus frusemide was consistent with no significant change in the plasma albumin. Similarly, there was no significant haemodilution with the alkali alone treatment.

The changes in fluid balance and weight were generally consistent with the changes in haematocrit and plasma albumin, particularly during the infusion.

(e) Summary and conclusions

Fluid balance and changes in weight and haemoconcentration were studied in 34 patients with aspirin poisoning. The patients were treated with either oral fluids only or one of the 4 intravenous regimes of fluid and alkali.

Haemodilution resulting from fluid retention was confirmed by changes in haematocrit and plasma albumin concentrations. The maximum fall in haematocrit and plasma albumin at the end of infusion occurred in the patients given forced alkaline diuresis and the minimum fall was observed in the patients given forced alkaline diuresis plus frusemide.

Haemodilution and fluid retention produced by the forced diuresis or forced alkaline diuresis regimes were confirmed by serial measurement of haematocrit, plasma albumin, patient weight and fluid balance. These changes were reduced by intravenous frusemide. However, the use of this diuretic did not enhance salicylate elimination (Chapter 2, Section IV). There was no significant haemodilution or fluid retention when alkali alone was given.

SECTION V

Chapter 2.

CHANGES IN PLASMA AND URINARY ELECTROLYTES AND CREATININE

(a) Introduction

The acute effects of therapeutic doses of acetylsalicylic acid on renal function have been studied in the dog (Berg and Bergan, 1976; Moncada et al., 1980) and in man (Berg, 1977a). The changes in plasma and urinary sodium and potassium concentrations in salicylate poisoning have been studied with different regimes of forced diuresis (Lawson et al., 1969), acetazolamid and sodium bicarbonate (Morgan and Polak, 1969), mannitol diuresis (Morgan, Bennett and Polak, 1968), and mannitol and forced alkaline diuresis (Prowse et al., 1970).

Lawson et al. (1969) found a fall in plasma sodium concentration only during the initial 2 hours in the patients treated by water diuresis, and a decline in plasma potassium concentration with forced alkaline diuresis, but the pattern of urinary excretion of sodium and potassium was similar despite the differences in the treatment regimes.

In the report of Morgan et al. (1968) plasma sodium concentrations ranged from 93 to 143 mmol/l and they recommended the addition of 20 mmol of potassium to each litre of intravenous fluid in salicylate poisoning. Prowse et al. (1970) observed falls in serum sodium, potassium, chloride and calcium concentrations in both groups, but a greater fall in plasma sodium was found in patients treated with mannitol. A greater fall was observed in plasma potassium concentrations in patients who received forced alkaline diuresis.

This /

This study was undertaken to monitor the effects of treatment on plasma and urinary electrolyte concentrations, osmolality and creatinine clearance in aspirin poisoning.

(b) Patients and methods

Six patients each of the control, forced diuresis, alkali alone, forced alkaline diuresis and forced alkaline diuresis with frusemide groups (Section IV) were studied. The amounts of electrolytes administered in the different treatment regimes are shown in Table 5.7.

Blood samples (10 ml) were taken in lithium heparin tubes on admission, at the end of infusion (at 4 hours after admission for the control group) and 16 hours following admission. The plasma was separated immediately and stored at -20°C. The urine samples were collected as described in Chapter 1, Section IV and 20 ml aliquots of each without preservative were placed in plain tubes and stored at -20°C.

The plasma electrolyte and creatinine concentrations were measured by Technicon Sequential Multiple Analysis plus Computer (SMAC) system, urinary electrolytes by flame photometer (Instrumentation Laboratory Inc Model IL 543), magnesium by atomic absorption (Instrumentation Laboratory Inc. Model IL353), plasma and urine osmolality by Advanced Digimatic Osmometer Model 3D II, and urine creatinine by Beckman Creatinine Analyser 2, in the University Department of Clinical Chemistry, Royal Infirmary, Edinburgh.

The paired two tailed Student t-test was used for the comparison within the groups and the independent two tailed Student t-test for the comparison between the groups.

(c) /

TABLE 5.7.

ELECTROLYTES ADMINISTERED IN THE DIFFERENT TREATMENT REGIMES (mmol)

Electrolyte	Forced diuresis	Alkali alone	Forced alkaline diuresis (FAD)	FAD + frusemide
Sodium	231	255	486	486
Potassium	120	60	120	120
Chloride	351	60	351	351
Bicarbonate	0	255	255	255

(c) Results

Plasma electrolytes and osmolality

The means and standard deviations of plasma electrolyte concentrations and osmolality on admission, at the end of infusion (4 hours for the control) and at 16 hours after admission, for each group of patients are given in Tables 5.8. - 5.13.

Plasma sodium

The plasma sodium concentrations did not change significantly during or after the infusion in any group (Table 5.8.). Although the concentrations declined during the infusion in the forced diuresis and forced alkaline diuresis with frusemide groups (135 and 134 mmol/l respectively), they were in the normal range (132-144 mmol/l). The plasma sodium concentration increased in the alkali alone regime during infusion and the difference between the changes in this and the forced diuresis group was significant ($p < 0.02$). The mean values at 16 hours following admission were 142, 141, 140, 139 and 138 mmol/l in the forced alkali diuresis, alkali alone, forced diuresis, forced alkaline diuresis plus frusemide and control groups respectively.

Plasma potassium

The plasma potassium concentrations also did not change significantly during or after the infusion (Table 5.9.). The maximum fall occurred with the forced alkaline diuresis regime from 4.2 to 3.5 mmol/l (normal range 3.3 - 4.7 mmol/l), but the mean concentration was higher on the following day (3.7 mmol/l). The mean values at 16 hours following admission were 4.0, 3.8, 3.7, 3.7 and 3.5 mmol/l with forced diuresis, control, alkali alone, forced alkaline diuresis and forced /

TABLE 5.8. CHANGES IN PLASMA SODIUM CONCENTRATIONS (mmol/l)* IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENTS FOR ASPIRIN POISONING

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	143 ± 3**	140 ± 3	139 ± 3	142 ± 2	142 ± 4
End of infusion	142 ± 2***	139 ± 5	134 ± 4	135 ± 6	144 ± 4
No. of patients with abnormally low concentrations at the end of infusion	0	1 (17%)	4 (67%)	2 (33%)	0
15 hr after start of infusion	138 ± 8****	142 ± 4	139 ± 2	140 ± 6	141 ± 2

None of the differences were statistically significant.

* Normal range 132 - 144 mmol/l. ** On admission. *** 4 hr after admission. **** 16 hr after admission.

TABLE 5.9. CHANGES IN PLASMA POTASSIUM CONCENTRATIONS (mmol/l)* IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENT FOR ASPIRIN POISONING

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	5.2 ± 2**	4.2 ± 1.0	4.2 ± 0.7	4.5 ± 0.4	5.1 ± 2.0
End of infusion	4.6 ± 0.9***	3.5 ± 0.6	3.8 ± 0.5	5.0 ± 0.9	4.7 ± 1.2
No. of patients with abnormally low concentrations at the end of infusion	1 (17%)	5 (83%)	3 (50%)	0	0
15 hr after start of infusion	3.8 ± 0.2****	3.7 ± 0.6	3.5 ± 0.7	4.0 ± 0.4	3.7 ± 0.4

None of the differences were statistically significant.

* Normal range 3.3 - 4.7 mmol/l. ** On admission. *** 4 hr after admission. **** 16 hr after admission.

TABLE 5.10. CHANGES IN PLASMA CALCIUM CONCENTRATIONS (mmol/l)* IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENTS FOR ASPIRIN POISONING

	Control (N = 6)	Forced alkaline diuresis (FAD)	FAD + frusemide (N = 6)	Forced diuresis (N = 6)	Alkali alone (N = 6)
Before infusion	2.60 ± 0.30***	2.40 ± 0.20	2.32 ± 0.20	2.43 ± 0.22	2.54 ± 0.26
End of infusion	2.30 ± 0.20****	2.03 ± 0.20	2.00 ± 0.14	2.07 ± 0.09	2.22 ± 0.20
No. of patients with abnormally low concentrations at the end of infusion	1 (17%)****	4 (67%)	4 (67%)	4 (67%)	1 (17%)
15 hr after start of infusion	2.20 ± 0.20*****	2.31 ± 0.18	2.24 ± 0.15	2.30 ± 0.31	2.30 ± 0.07
p - value**	< 0.01	< 0.005	< 0.005	< 0.02	< 0.001

* Normal range 2.12 - 2.62 mmol/l. ** Before compared with the end of infusion. *** On admission.

**** 4 hr after admission. ***** 16 hr after admission.

TABLE 5.11. CHANGES IN PLASMA PHOSPHATE CONCENTRATIONS (mmol/l)* IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENT FOR ASPIRIN POISONING

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	1.20 ± 0.17***	1.24 ± 0.22	1.08 ± 0.30	1.24 ± 0.20	1.20 ± 0.13
End of infusion	1.31 ± 0.20****	0.65 ± 0.30	0.65 ± 0.33	0.82 ± 0.20	1.13 ± 0.22
No. of patients with abnormally low concentrations at the end of infusion	0	4 (67%)	4 (67%)	2 (33%)	0
15 hr after start of infusion	0.80 ± 0.24*****	0.72 ± 0.11	0.69 ± 0.25	0.68 ± 0.12	0.84 ± 0.21
p - value**	> 0.60	< 0.01	< 0.025	< 0.05	> 0.30

* Normal range 0.80 - 1.40 mmol/l. ** Before compared with the end of infusion. *** On admission.

**** 4 hr after admission. ***** 16 hr after admission.

TABLE 5.12. CHANGES IN PLASMA MAGNESIUM CONCENTRATIONS (mmol/l)* IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENT FOR ASPIRIN POISONING

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	0.87 ± 0.06***	0.87 ± 0.11	0.90 ± 0.10	0.88 ± 0.13	0.90 ± 0.11
End of infusion	0.83 ± 0.09****	0.74 ± 0.10	0.66 ± 0.13	0.75 ± 0.11	0.79 ± 0.07
No. of patients with abnormally low concentrations at the end of infusion	1 (17%)	3 (50%)	4 (67%)	3 (50%)	1 (17%)
15 hr after start of infusion	0.82 ± 0.09*****	0.82 ± 0.12	0.84 ± 0.10	0.77 ± 0.1	0.84 ± 0.08
p - value **	> 0.05	< 0.001	< 0.01	< 0.02	< 0.02

* Normal range 0.75 - 1.0 mmol/l. ** Before compared with the end of infusion. *** On admission.
**** 4 hr after admission. ***** 16 hr after admission

TABLE 5.13. CHANGES IN PLASMA OSMOLALITY (mmol/kg*) IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENT FOR ASPIRIN POISONING

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	308 ± 26***	292 ± 6	311 ± 23	294 ± 24	305 ± 22
End of infusion	295 ± 0.18****	286 ± 2	289 ± 5	275 ± 16	296 ± 13
No. of patients with abnormally low concentrations at the end of infusion	0	0	0	4 (67%)	0
15 hr after start of infusion	287 ± 4*****	288 ± 8.4	281 ± 9	278 ± 13	282 ± 4
p - value**	> 0.05	> 0.05	> 0.05	< 0.02	> 0.05

* Normal range 280-290 mmol/kg. ** Before compared with the end of infusion. *** On admission.

**** 4 hr after admission. ***** 16 hr after admission.

forced alkaline diuresis plus frusemide groups respectively. There were no statistically significant differences between the plasma potassium concentrations in the different treatment groups.

Plasma calcium

The plasma calcium concentrations on admission were in the normal range (2.12 - 2.62 mmol/l) in all patients, but decreased significantly 4 hours later in all groups including the control (Table 5.10.). The mean values at the end of infusion were below the minimum normal in the forced diuresis (2.07), forced alkaline diuresis (2.03) and forced alkaline diuresis with frusemide (2.00 mmol/l) groups. However, mean plasma calcium concentrations returned to the normal range at 16 hours following admission (Fig. 5.3.).

Plasma phosphate

The plasma phosphate concentrations on admission were normal (0.8 - 1.4 mmol/l) in all patients except for one (No. 57) treated with forced alkaline diuresis plus frusemide in whom the concentration declined from 0.72 to 0.34 mmol/l during the infusion. This patient was a 24 year old woman with acute on chronic aspirin poisoning in whom vagotomy had been carried out 8 months before admission because of gastric ulcer.

The mean plasma phosphate concentrations decreased significantly during the infusion in the forced diuresis, forced alkaline diuresis and forced alkaline diuresis plus frusemide groups ($p < 0.05$, $p < 0.01$ and $p < 0.025$ respectively (Table 5.11.). The mean values at the end of infusion were 0.82, 0.65 and 0.65 mmol/l respectively (Fig. 5.3.). The /

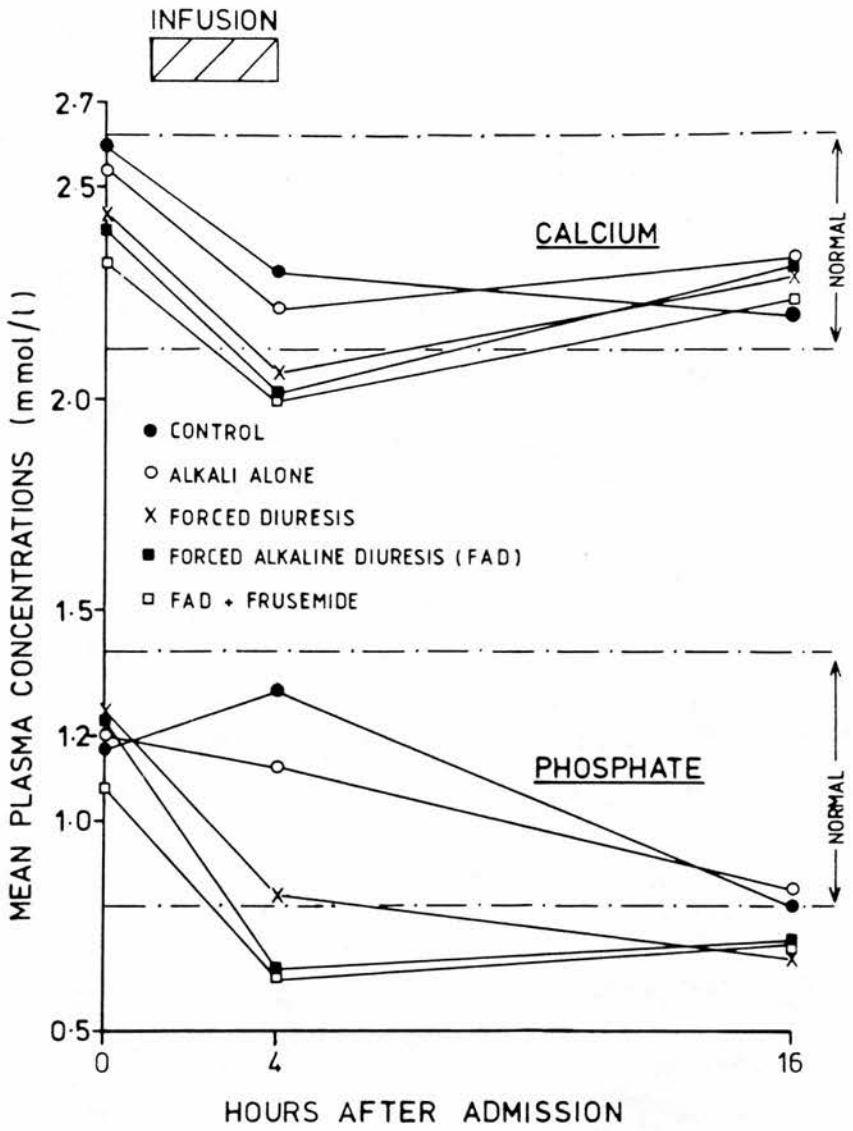

Figure 5.3. Changes in plasma concentrations of calcium and phosphate in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

The mean concentration decreased from 1.20 to 1.13 mmol/l in the alkali alone group and increased from 1.20 to 1.31 mmol/l in the control group. The differences between the changes in the latter two groups and the former three were highly significant ($p < 0.01$ to $p < 0.001$). The mean plasma phosphate concentrations at 16 hours after admission were 0.84, 0.80, 0.72, 0.69 and 0.68 mmol/l in the alkali alone, control, forced alkaline diuresis, forced alkaline diuresis plus frusemide and forced diuresis groups respectively.

Plasma magnesium

The plasma magnesium concentrations on admission were all normal (0.75 to 1.00 mmol/l), but decreased significantly during the infusion in all treated groups (Table 5.12. and Fig. 5.4.). The mean values at the end of infusion were 0.83, 0.79, 0.75, 0.74 and 0.66 mmol/l in the control, alkali alone, forced diuresis, forced alkaline diuresis and forced alkaline diuresis plus frusemide groups respectively. The changes during infusion were significantly greater in the treated than in the control groups ($p < 0.01$). The concentrations returned to normal at 16 hours after admission.

Plasma osmolality

The plasma osmolality on admission was above the normal range (280 - 290 mmol/kg) in all patients, but it decreased during the infusion. In the forced diuresis group the decline was significant ($p < 0.02$) and below the minimum normal (280 mmol/kg) (Fig. 5.4.). The mean values at the end of infusion were 296, 295, 289, 286 and 275 mmol/kg in the alkali alone, control, forced alkaline diuresis with /

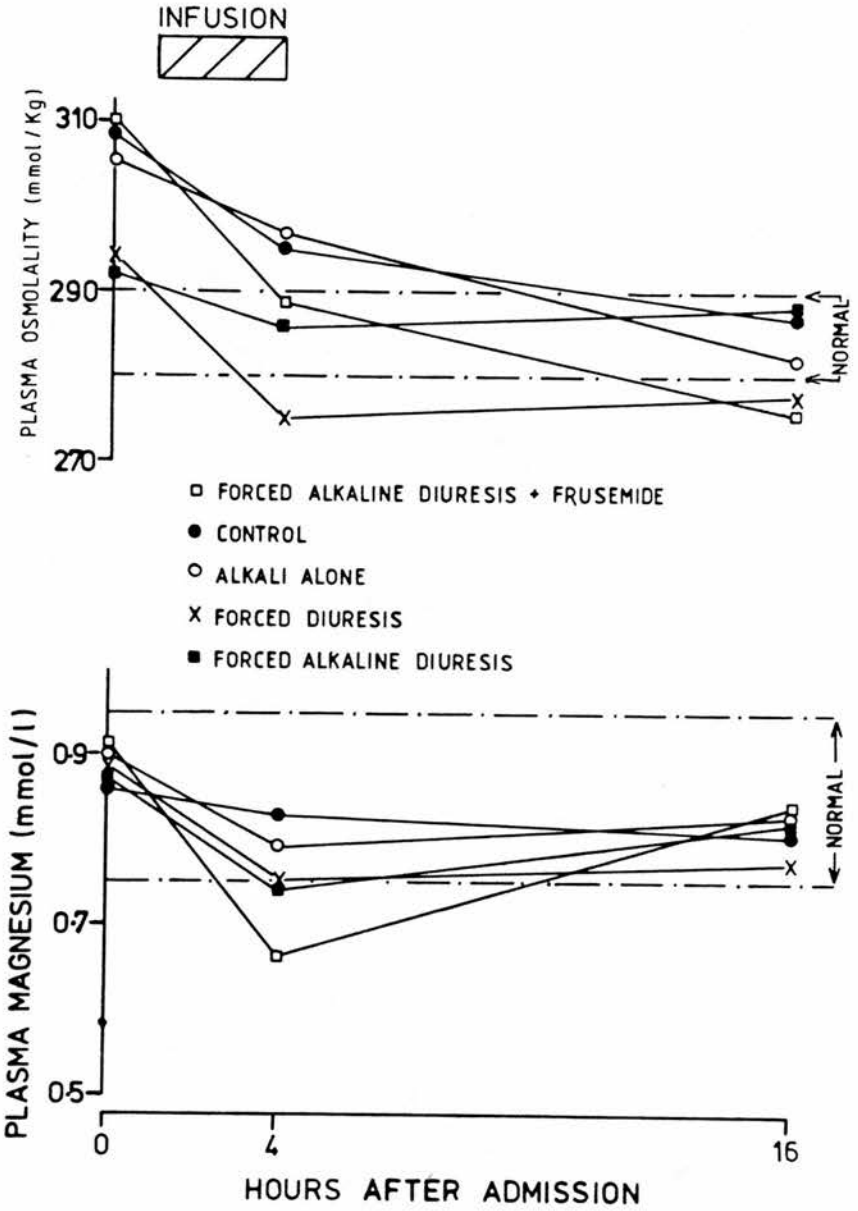

Figure 5.4. Changes in plasma osmolality and magnesium concentrations in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

with frusemide, forced alkaline diuresis and forced diuresis groups respectively (Table 5.13.). At the end of infusion the plasma osmolality was significantly higher in the alkali alone than in the forced diuresis group ($p < 0.05$). There were no statistically significant differences between the groups at 16 hours after admission, although the mean plasma osmolality was below the normal range in the forced diuresis group (278 mmol/kg).

Urinary sodium potassium and osmolality

The means and standard deviations of urinary sodium and potassium concentrations and osmolality and the sodium/potassiom ratio for each group are shown in Table 5.14.

The urinary sodium concentration was less than 47 mmol/24 hr (normal range 100 - 200 mmol/24 hr) in all control patients except one (No. 43). The mean urinary sodium concentration was significantly higher in the treated groups than in the controls ($p < 0.005$). The mean urinary sodium excretion was 474, 272, 257, 212 and 50 mmol/24 hr in the forced alkaline diuresis plus frusemide, alkali alone, forced alkaline diuresis, forced diuresis and control groups, respectively. There were no statistically significant differences between the forced diuresis, forced alkaline diuresis and alkali alone groups, but sodium excretion was significantly lower than in the forced alkaline diuresis with frusemide regime ($p < 0.001$, $p < 0.02$ and $p < 0.005$ respectively).

Urine potassium

The urinary potassium was in the normal range (25 - 100 mmol/24 hr) in all control patients, and only in 4 patients of the treated groups. /

TABLE 5.14. URINARY SODIUM, POTASSIUM AND OSMOLALITY IN GROUPS OF 6 PATIENTS FOLLOWING DIFFERENT TREATMENTS FOR ASPIRIN POISONING

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Urinary sodium (mmol/24 hr)*	50 ± 35	257 ± 136	474 ± 121	212 ± 58	272 ± 45
Urinary potassium (mmol/24 hr) **	51 ± 26	148 ± 94	179 ± 77	145 ± 48	124 ± 41
Urinary sodium/ potassium ratio ***	1.24 ± 0.89	2.04 ± 1.13	3.03 ± 1.35	1.15 ± 0.26	2.40 ± 0.93
Urinary osmolality (mmol/kg)	467 ± 132	330 ± 114	354 ± 77	298 ± 63	420 ± 56

* Normal range 100-200 mmol/ 24 hr. ** Normal range 25-100 mmol/ 24 hr.

*** The ratios were calculated for individual data and therefore the mean values do not correspond.

groups. The 24 hour potassium excretion in the treated groups was significantly higher than in the controls ($p < 0.005$). The mean values were 179, 148, 145, 124 and 51 mmol/24 hr in the forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis, alkali alone and control groups respectively. There were no statistically significant differences between the treated groups.

Urine sodium/potassium ratio

The mean ratios of urinary sodium to potassium over the 24 hours were 3.03, 2.40, 2.04, 1.24 and 1.15 in the forced alkaline diuresis plus frusemide, alkali alone, forced alkaline diuresis, control and forced diuresis groups respectively. There were no statistically significant differences between the forced diuresis, forced alkaline diuresis and control groups, but the ratio in the forced diuresis group was significantly lower than in the alkali alone and forced alkaline diuresis with frusemide groups ($p < 0.01$). The ratio in the control group was also significantly lower than in the forced alkaline diuresis plus frusemide group ($p < 0.01$).

Urine osmolality

The mean values of urinary osmolality were 467, 420, 354, 330 and 298 mmol/kg in the control, alkali alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis and forced diuresis groups respectively. There were no statistically significant differences between the groups.

Creatinine clearance

The creatinine clearance over the whole period of study for each patient /

patient is shown in Table 5.15. The values varied from 18 ml/min in a control patient (No. 36) to 170 ml/min in a patient who received forced diuresis. The mean creatinine clearances were 105, 99, 98, 87 and 79 ml/min with forced alkaline diuresis plus frusemide, forced diuresis, alkali alone, forced alkaline diuresis and control groups respectively. There were no statistically significant differences between the groups. Half the patients (15) had creatinine clearance less than 100 ml/min with no marked differences between the groups.

There were no statistically significant correlations between creatinine clearance and the renal clearance of salicylic and salicyluric acids, the plasma salicylic acid half-life, urine pH and flow rate, urinary sodium or potassium excretion or the ratio of urinary sodium to potassium.

(d) Discussion

Although different amounts of sodium, potassium and chloride were administered intravenously in the different regimes, the plasma sodium and potassium concentrations did not differ significantly either at the end of infusion or 12 hours later. These findings are in good agreement with those of Morgan et al. (1968), Lawson et al. (1969) and Berg (1977) except that hypokalaemia occurred with the forced alkaline diuresis regime of Lawson et al. (1969) in which administration of potassium was delayed.

The significant fall in plasma calcium concentrations during infusion is consistent with the findings of Prowse et al. (1970), but since it does not correspond to the fall in haematocrit, it cannot be attributed to haemodilution only as they claimed. Furthermore, a similar fall was observed in the control patients (Fig. 5.3.). However, /

TABLE 5.15. CREATININE CLEARANCE (ml/min) IN PATIENTS WITH MILD TO SEVERE ASPIRIN POISONING RECEIVING DIFFERENT TREATMENTS

Control		Forced alkaline diuresis (FAD)		FAD + frusemide		Forced diuresis		Alkali alone	
No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min	No.	ml/min
36	18	20	71	53	112	27	170	37	49
43	117	22	103	54	103	29	67	38	131
45	83	24	108	55	118	30	109	44	105
48	132	24	101	57	83	31	110	47	123
50	75	26	97	58	125	32	61	49	98
52	46	41	44	59	87	33	75	51	84
Mean:	79		87		105		99		98
S.D.:	43		25		17		41		30

Analysis of variance: F - Ratio = 0.62 $p > 0.05$

However, there was no obvious clinical evidence of hypocalcaemia and the concentrations returned to normal without calcium supplements.

It is of interest that the fall in plasma phosphate concentrations was significant with forced alkaline diuresis and forced diuresis, but not with alkali alone. This fall is far too great to be accounted for by haemodilution. It is associated with salicylate intoxication per se, since it occurred in the controls, and infusion of dextrose itself may cause a marked fall in plasma phosphate (Guillou, Morgan and Hill, 1976).

Changes in plasma magnesium concentration, except in the report by Morgan and Polak (1969) when it was mentioned as normal, have not been reported in aspirin poisoning. The fall was not statistically significant in the control patients and it could be due largely to haemodilution.

The high plasma osmolality on admission in all groups and the significant fall with forced diuresis and forced alkaline diuresis are consistent with previous reports (Morgan et al., 1968; Berg, 1977b).

The low urinary sodium output in the control patients confirmed the study of Berg (1977a). Only with alkali alone was the 24 hour urinary sodium excretion (272 mmol) greater than the amount infused (255 mmol), and even the highest sodium output with forced alkaline diuresis plus frusemide (474 mmol) was less than the amount infused (486 mmol). In addition, oral fluids also contained sodium which must be taken into account. Thus there is no doubt that sodium is retained, presumably as the result of the inhibitory effects of aspirin on renal prostaglandin synthesis (Plotz and Kimberly, 1981).

The urinary excretion of potassium was abnormally high in all the /

the different groups. The low ratio of urinary sodium to potassium with the control and forced diuresis groups was as described by Berg (1977a), but the higher ratios with forced alkaline diuresis are presumably due to higher sodium content of the infusion fluids. The higher ratio with the alkali alone treatment is probably due to the smaller amount of potassium administered.

The creatinine clearance was surprisingly low in half of the patients, which is consistent with the observations of Muther and Bennett (1980), and the nephrotoxicity of aspirin (Prescott, 1965, 1976 and 1979c; Kimberly and Plotz, 1981). The lack of correlation between the creatinine clearance and renal clearance of salicylic acid was also observed by Morgan and Polak (1971). The very low creatinine clearance associated with urine flow rates of less than 0.5 ml/min in normal subjects found by Chesley (1938) did not apply to the overdose patients with low creatinine clearance.

(e) Summary and conclusions

Changes in plasma and urinary electrolyte concentrations, osmolality and creatinine clearance were studied in 30 patients with mild to severe aspirin poisoning. The patients were treated with either oral fluids or one of the 4 intravenous regimes of fluid and alkali.

The plasma sodium and potassium concentrations did not change significantly during or after infusion and there were no statistically significant differences between the groups, although the amounts of electrolytes infused were not the same.

The plasma calcium concentrations on admission were normal in all patients, but decreased significantly 4 hours later in all groups. There were no hypocalcaemic symptoms and the concentrations returned to normal 16 hours after admission without administration of calcium.

The /

The plasma phosphate concentrations on admission were also normal, but decreased significantly during infusion with the forced diuresis and forced alkaline diuresis regimes, but not in the alkali alone and control groups. The concentrations returned to normal 16 hours after admission in all except in the forced diuresis group in which the mean plasma phosphate concentration was 0.68 mmol/l.

The plasma magnesium concentrations on admission were all normal, but decreased significantly during infusion in all treated groups, but not in the controls. The concentrations returned to normal at 16 hours in all groups.

The mean plasma osmolality on admission was abnormally high in all groups, but declined significantly with forced diuresis treatment to below the minimum normal at the end of infusion (275 mmol/kg) and 12 hours later (278 mmol/kg).

The urinary sodium excretion was very low in the control patients (50 mmol/24 hr), but not in the treated groups because of sodium infusion. The highest sodium output was obtained with forced alkaline diuresis plus frusemide (474 mmol/24 hr), but this was still less than the amount of sodium infused (486 mmol/kg). Therefore sodium was retained probably as a result of renal prostaglandin synthetase inhibition by aspirin.

The urinary potassium excretion was normal in the control patients and increased in the treated groups presumably because of potassium supplements in the infusion fluids. Urinary osmolality was similar in all groups.

The creatinine clearance was very low in some patients, and this no doubt reflects the known nephrotoxic effects of salicylates. There were no statistically significant differences between the groups.

The /

The alkali alone regime had the least effect on the plasma and urinary electrolytes, and forced diuresis showed the maximum disturbances in comparison with the other treatments.

SECTION VI

CLINICAL, BIOCHEMICAL AND HAEMATOLOGICAL STUDIES IN ACETYLSALICYLIC ACID POISONING

SECTION VI

Chapter 1.

CLINICAL AND BIOCHEMICAL ABNORMALITIES IN ACETYLSALICYLIC ACID POISONING AND THE EFFECTS OF TREATMENT WITH FLUID AND ALKALI

(a) Introduction

Although the clinical features of salicylate poisoning have been well documented (Done, 1960; Cumming, 1961; Beveridge et al., 1964; Bender, 1975; Anderson et al., 1976; Proudfoot and Prescott, 1977; Temple, 1978; Matthew and Lawson, 1979), their frequency has not been reported in detail. Only in one report (Beveridge et al., 1964) were the clinical features of 18 patients with aspirin poisoning documented for each patient separately.

Biochemical abnormalities such as acid-base disturbances (Hyder et al., 1945; Winters et al., 1959; Proudfoot and Brown, 1969; Gabow et al., 1978) and electrolyte abnormalities (Lawson et al., 1969; Morgan and Polak, 1971; Berg, 1977b) have been investigated. However, metabolic abnormalities related to salicylate poisoning such as changes in lactate and pyruvate metabolism have not been reported.

The present study was undertaken to document the clinical features and to investigate possible changes in plasma total bicarbonate, urate, urea, proteins, lactate and pyruvate concentrations together with liver function tests in patients with aspirin poisoning.

(b) Patients and methods

The clinical features in 55 patients with aspirin poisoning who were /

were treated with different regimes of fluid and alkali were recorded. Eighteen patients (9 males and 9 females) were treated with standard forced alkaline diuresis, 6 (3 males and 3 females) with forced diuresis, 6 (3 males and 3 females) with forced alkaline diuresis plus frusemide, 7 (3 males and 4 females) with alkali alone and 18 controls (8 males and 10 females) with oral fluids only (Section IV). Clinical findings were recorded during the first 4 hours and at 12 hours after admission. The patients were questioned for the presence of tinnitus, deafness and other symptoms such as epigastric pain. Physical examination was performed on admission, at the end of infusion (4 hours after admission for the control patients) and at 12 hours following admission. The pulse rate, temperature, respiration rate and blood pressure were measured hourly for 12 hours.

The plasma total CO_2, urate, urea, and total protein concentrations and liver function tests were estimated by the Technicon Sequential Multiple Analysis plus Computer (SMAC) System in groups of 6 patients as described in Chapter 2, Section V. Plasma lactate and pyruvate concentrations were measured in 6 patients each of the control and forced alkaline diuresis groups. The venous blood samples (2 ml) for lactate measurement were placed into tubes containing 80 µl fluoride reagent and centrifuged for 5 minutes at 3000 rpm within 2 hours. The lactate concentration in the supernatant was measured by Rotochem II Aminco, using a Boehringer kit (Product No. 149993). For pyruvate measurements, blood (2 ml) was placed in tubes containing 4 ml of 5% perchloric acid. After mixing the tube was centrifuged immediately and the pyruvate concentration of the supernatant measured using a Sigma pyruvic acid kit (No. 726-10). The biochemical tests were all performed in the University Department of Clinical Chemistry at the Royal Infirmary of Edinburgh.

(c) /

(c) Results

Clinical findings

The clinical findings during the first 4 hours after admission are expressed as the percentage of patients with positive symptoms and signs of poisoning in Table 6.1. The mean peak plasma salicylic acid concentrations in each group are also shown.

Tinnitus was the commonest symptom in all groups and occurred in 78% of the control patients, 94% of the patients who received the forced alkaline diuresis and in all patients in the other 3 groups. There was a clear relationship between the peak plasma salicylic acid concentrations and tinnitus. Only one patient in the forced alkaline diuresis group who had a low plasma salicylic acid concentration (271 µg/ml) did not complain of tinnitus. Four control patients with peak plasma salicylate concentrations of 277 to 391 µg/ml also denied tinnitus. However, 91% of the 55 patients who had plasma salicylic acid concentrations of 260 - 657 µg/ml complained of this symptom. Subjective loss of hearing was the second commonest symptom of aspirin poisoning and occurred in 83% of the patients treated with forced alkaline diuresis, forced alkaline diuresis plus frusemide and forced diuresis, in 50% of the control patients and in 43% of those treated with alkali alone.

Sweating was present in 56% of all patients, and the incidence varied from 33% in the forced diuresis group to 83% in the forced alkaline diuresis plus frusemide group. Hyperventilation (respiration rate more than 20 per minute) was recorded in 86, 67, 61, 50 and 22 percent in the alkali alone, forced alkaline diuresis plus frusemide, forced alkaline diuresis, forced diuresis and control groups respectively. Nausea, vomiting and epigastric pain or tenderness were noted in /

TABLE 6.1. PERCENTAGE OF PATIENTS WITH POSITIVE CLINICAL FEATURES OF ASPIRIN POISONING DURING THE FIRST 4 HOURS AFTER ADMISSION

Group	Peak plasma salicylic acid concentration (μg/ml)	Tinnitus	Deafness	Sweating	Hyper-ventil-ation	Nausea	Vomiting	Epigastric pain/ tenderness	Flushing
Control (N = 18)	342 ± 57	78	50	44	22	39	28	39	28
Forced alkaline diuresis (FAD) (N = 18)	474 ± 99	94	83	66	61	56	50	28	39
FAD + frusemide (N = 6)	465 ± 83	100	83	83	67	67	67	67	67
Forced diuresis (N = 6)	471 ± 63	100	83	33	50	67	50	50	17
Alkali alone (N = 7)	449 + 74	100	43	57	86	14	0	0	14
Overall (N = 55)	426 ± 97	91	63	56	51	43	38	34	33

in 43, 38 and 34% of patients respectively. Flushing was also present in 33% of all patients (Table 6.1.).

The clinical findings 12 hours after admission in the same patients are given in Table 6.2. Tinnitus was still present in 45% of all patients, but varied with different treatments. The lowest frequency (28%) was found in the alkali alone and forced alkaline diuresis groups and the highest (67%) in the control patients. Tinnitus was also present in 50% of the patients treated by forced diuresis, forced alkaline diuresis with frusemide. Deafness was still present 12 hours after admission in 17, 11 and 6% of the patients in the forced diuresis, control and forced alkaline diuresis groups respectively, but in none of those in the alkali alone and forced alkaline diuresis with frusemide groups. Sweating, hyperventilation and flushing had disappeared in all patients 12 hours after admission. Pyrexia was noted in 6 severely poisoned patients and tachycardia (< 130 per minute) in 14 patients who received 6 litres of fluid over 3 hours.

All patients recovered uneventfully and were discharged 16 to 92 hours after admission.

Biochemical abnormalities

Plasma total CO_2

Plasma total CO_2 concentrations on admission were below normal (24 - 30 mmol/l) in 22 out of the 30 patients (Table 6.3.).

The plasma total CO_2 concentrations increased significantly during infusion with the alkalinisation groups ($p < 0.01$ to $p < 0.005$) and decreased significantly in the forced diuresis and control groups ($p < 0.01$ and $p < 0.05$ respectively) (Fig. 6.1.). The mean values at the /

TABLE 6.2.

PERCENTAGE OF PATIENTS WITH POSITIVE CLINICAL FEATURES OF ASPIRIN POISONING 12 HOURS AFTER ADMISSION

Group	Plasma salicylic acid concentration at 12 hours	Tinnitus	Deafness	Sweating	Hyper-ventil-ation	Nausea	Vomiting	Epigastric pain/ tenderness	Flushing
Control (N = 18)	249 ± 50	67	11	0	0	0	0	0	0
Forced alkaline diuresis (FAD) (N = 18)	154 ± 95	28	6	0	0	0	0	0	0
FAD + frusemide (N = 6)	215 ± 47	50	0	0	0	50	0	33	0
Forced diuresis (N = 6)	224 ± 54	50	17	0	0	17	0	0	0
Alkali alone (N = 7)	134 ± 83	28	0	0	0	0	0	0	0
Overall (N = 55)	198 ± 89	45	7.3	0	0	7.3	0	3.6	0

TABLE 6.3.

PLASMA TOTAL CO2 CONCENTRATIONS (mmol/l) IN GROUPS OF 6 PATIENTS WITH ASPIRIN POISONING RECEIVING DIFFERENT TREATMENTS

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	23 ± 1**	21 ± 4	19 ± 4	24 ± 2	22 ± 4
End of infusion	21 ± 2***	26 ± 4	26 ± 3	19 ± 2	28 ± 3
No. of patients with abnormally low concentration at end of infusion	5	1	0	6	0
15 hours after the start of infusion	24 ± 2****	26 ± 2	27 ± 2	21 ± 1	25 ± 1
p - value *	< 0.05	< 0.01	< 0.025	< 0.01	< 0.005

Normal range : 24-30 mmol/l.

* Comparisons between the beginning and end of infusion. ** On admission. *** 4 hours after admission.
**** 16 hours after admission.

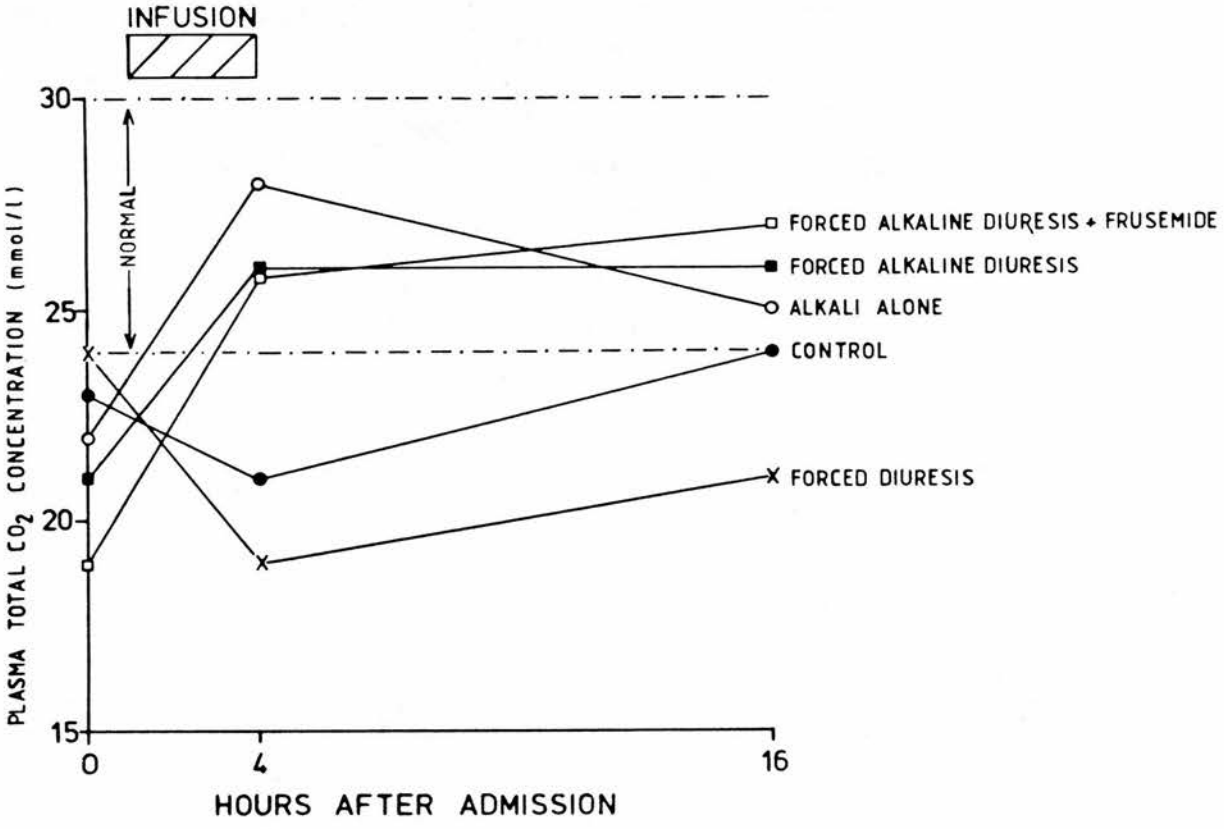

Figure 6.1. Changes in plasma total CO_2 concentrations in patients with mild to severe aspirin poisoning receiving different treatments of fluid and alkali.

the end of infusion (4 hours after admission for the controls) were 28, 26, 26, 21 and 19 mmol/l with alkali alone, forced alkaline diuresis, forced alkaline diuresis plus frusemide, control and forced diuresis groups respectively. There were no statistically significant differences between the alkalinisation groups, but the plasma total CO_2 concentrations were significantly higher in these than in the forced diuresis and control groups ($p < 0.01$ and $p < 0.05$ respectively).

The plasma total CO_2 concentrations at 16 hours after admission had returned almost to normal in the non-alkalinisation groups, but were still below normal in all the patients treated with forced diuresis and 4 control patients. The mean values were 27, 26, 25, 24 and 21 mmol/l with forced alkaline diuresis plus frusemide, forced alkaline diuresis, alkali alone, control and forced diuresis groups respectively. There were no statistically significant differences between the alkalinisation groups, but the mean plasma total CO_2 concentrations were higher in these than in the forced diuresis ($p < 0.001$) but not the control patients.

Plasma urate

The plasma urate concentration on admission was below the normal range (male 0.12 - 0.42, female 0.12 - 0.36 mmol/l) in 3 patients, one control and 2 given forced alkaline diuresis with frusemide. The concentrations declined significantly during the first 4 hours after admission in all groups including the control. The fall was most marked in the diuresis groups (Table 6.4.). The mean plasma urate concentrations declined further subsequently, particularly in the alkaline alone and control groups and by 16 hours the concentrations in all groups were similar. The initial rapid fall with the diuretic regimes presumably reflected haemodilution.

Plasma /

TABLE 6.4.

PLASMA URATE CONCENTRATIONS (mmol/l) IN GROUPS OF 6 PATIENTS WITH ASPIRIN POISONING RECEIVING DIFFERENT TREATMENTS

	Control	Forced alkaline diuresis (FAD)	FAD + frusemide	Forced diuresis	Alkali alone
Before infusion	0.24 ± 0.09**	0.22 ± 0.05	0.23 ± 0.13	0.25 ± 0.09	0.26 ±0.06
End of infusion	0.20 ± 0.07***	0.13 ± 0.03	0.16 ± 0.10	0.16 ± 0.06	0.20 ± 0.05
No. of patients with abnormally low concentration at the end of infusion	0	2	3	2	0
15 hours after the start of infusion	0.15 ± 0.06****	0.13 ± 0.03	0.16 ± 0.09	0.14 ± 0.05	0.17 ± 0.05
p - value *	< 0.05	< 0.001	< 0.05	< 0.02	< 0.001

Normal range: male 0.12 - 0.42, female 0.12 - 0.36 mmol/l.

* Before comparison with the end of infusion. ** On admission. *** 4 hours after admission.

**** 16 hours after admission.

Plasma lactate and pyruvate

The plasma lactate concentrations were in the normal range (0.63 - 2.44 mmol/l) in all control patients and those treated by forced alkaline diuresis with no statistically significant difference between them (Table 6.5.). The mean values were 1.18 and 1.12 mmol/l respectively.

The plasma pyruvate concentrations were above the normal range (35 - 80 μmol/l) in 3 control patients and 2 with the forced alkaline diuresis regime (Table 6.5.). The mean values were 91 and 79 μmol/l respectively and did not differ significantly. There was no statistically significant correlation between pyruvate and the corresponding plasma salicylic acid concentrations.

Other biochemical measurements

The plasma concentrations of urea, bilirubin, alkaline phosphatase, alanine and aspartate aminotransferases, and urea stable lactate dehydrogenase were normal in all patients and did not change significantly during or after infusion, except for urea which declined significantly from a mean of 4.05 to 3.32 mmol/l during infusion ($p < 0.05$) in the forced diuresis group.

The changes in total plasma protein concentration were similar to those observed with plasma albumin as described in Chapter 1, Section V..

(d) Discussion

The main clinical manifestations of salicylate poisoning in adults are tinnitus, deafness, sweating, hyperventilation, flushing and /

TABLE 6.5.

PLASMA LACTATE AND PYRUVATE CONCENTRATION IN PATIENTS WITH ASPIRIN POISONING

Treatment	Patient No.	Hours after ingestion	Lactate (mmol/l)*	Pyruvate (μmol/l)**	Plasma salicylic acid concentration (μg/ml)
Control	1	11½	1.55	130	355
	1	34½	1.51	145	163
	6	16½	1.41	128	205
	7	17½	1.12	46	170
	8	23½	1.16	46	231
	9	24¾	0.80	116	233
	13	16½	0.70	29	227
Mean ± S.D.	-	20.7 ± 7.6	1.18 ± 0.34	91.4 ± 49	226 ± 64
Forced alkaline diuresis	2	17¼	0.78	75	101
	5	28½	1·16	77	152
	10	31½	0.95	94	251
	11	47	1.45	49	67
	12	21½	0.59	108	167
	15	39	1.77	70	2.8
Mean ± S.D.	-	30.8 ± 11.0	1.12 ± 0.44	79 ± 20	123 ± 86

* Normal range 0.63-2.44 mmol/l. ** Normal range 35-80 μmol/l.

and epigastric pain (Beveridge et al., 1964; Proudfoot and Prescott, 1977; Matthew and Lawson, 1979). Loss of consciousness is very rare in adults and when it does occur, intoxication is severe and likely to be fatal (Proudfoot and Prescott, 1977).

The correlation between tinnitus and plasma salicylic acid concentrations is in agreement with the findings of Beveridge et al. (1964), but deafness occurred in 63% of 55 patients in the present study compared with 100% of the 18 patients they studied. The frequencies of sweating, hyperventilation and epigastric pain were similar to those observed by Beveridge et al. (1964), but they did not mention nausea, vomiting and gastric haemorrhage. Mongan et al. (1973) reported tinnitus in 59 rheumatic patients who had serum salicylate concentrations over 196 μg/ml (average 304 μg/ml), but Seltzer (1973) has described tinnitus, deafness and vertigo after ingestion of only 2 plain aspirin tablets.

The more rapid relief of the symptoms of poisoning in patients who were treated by alkali alone is consistent with the more rapid elimination of salicylic acid in this group. The reduced efficacy of the forced diuresis in enhancing salicylate elimination is also reflected in the persistence of symptoms of salicylism. The combination of frusemide and forced alkaline diuresis did not produce greater relief of symptoms and this is as expected from elimination kinetics.

The low plasma total CO_2 concentration on admission is presumably a response to hyperventilation rather than metabolic acidosis (Segar and Holliday, 1958; Temple, 1978). Again, as expected, concentrations rapidly returned to normal following the administration of alkali.

The /

The decrease in plasma urate concentrations is presumably due to the dose-dependent uricosuric effect of salicylate (Flower et al., 1980). Changes in plasma urate concentrations following aspirin overdosage have not been described previously.

The plasma lactate concentrations were within the normal range in all patients and the pyruvate concentrations were abnormally high in a minority of patients. There was no correlation between plasma pyruvate and salicylic acid concentrations. There has been no report of plasma lactate and pyruvate concentrations in aspirin overdosage, although the theory of the inhibitory effects of salicylate on the Krebs cycle dehydrogenases would be expected to increase the plasma lactic and pyruvic acid concentrations (Segar and Holliday, 1958; Temple, 1978).

The normal values for plasma bilirubin, alkaline phosphatase and aminotransferases throughout the study in all patients provided no evidence of acute salicylate-induced hepatotoxicity, but the time was not long enough in all cases to exclude hepatotoxicity. However, hepatotoxicity has been associated with chronic salicylate administration (Sharbaro and Bennett, 1977; Bernstein et al., 1977; Schaller, 1978; Kolling and Hinddin, 1978; Gullner, 1979b).

(e) Summary and conclusions

The clinical features in 55 adult patients with aspirin poisoning treated with oral fluids only or one of four intravenous regimes of fluid and alkali were recorded.

Plasma concentrations of total CO_2, urate, urea, were monitored together with liver function tests in 5 groups of 6 patients. Plasma lactate /

lactate and pyruvate concentrations were measured in the control and forced alkaline diuresis groups.

Tinnitus was the commonest symptom and was present in 91% of all patients. It correlated well with plasma salicylic acid concentrations. Subjective deafness occurred in 63% of the patients, while sweating, hyperventilation, nausea, vomiting, epigastric pain/tenderness and flushing were found in 56, 51, 43, 38, 34 and 33% of the patients respectively.

These manifestations of salicylism had disappeared in most patients 12 hours after admission. The most rapid improvement was obtained with the alkali alone regime and the least improvement occurred with forced diuresis.

The mean plasma total CO_2 concentrations on admission were below normal in all groups, but returned to normal within 4 hours in all the alkalinisation groups. There was a further decline in the forced diuresis and control groups.

The plasma urate concentrations decreased in almost all patients. The greatest fall occurred during the first 4 hours in the diuresis groups and this was due to haemodilution rather than the uricosuric effect of aspirin

The plasma lactate concentrations were normal in all patients and pyruvate concentrations were abnormally high in less than half.

There were no changes in the concentration of plasma urea, bilirubin, alkaline phosphatase and aminotransferases.

SECTION VI

Chapter 2

HAEMATOLOGICAL CHANGES AFTER THERAPEUTIC DOSAGE AND OVERDOSAGE OF ACETYLSALICYLIC ACID

(a) Review of the literature

The effects of acetylsalicylic acid on platelet function have been investigated for many years. Evans et al. (1968) studied the inhibitory effects of aspirin and sodium salicylate on platelet aggregation induced by collagen, antigen-antibody complex, gamma globulin coated particles and thrombin. They concluded that diminished aggregation was due to a defect in the release of adenosine diphosphate. Weiss, Aledort and Kochwa (1968) reported the effects of salicylates on the haemostatic properties of platelet in man. They stressed that the ingestion of aspirin, but not sodium salicylate, prolonged the bleeding time significantly and irreversibly impaired platelet aggregation with a decreased release of platelet adenosine diphosphate. O'Brien (1968) also demonstrated that adenosine diphosphate is not released by platelets exposed to aspirin (but not to sodium salicylate) and concluded that some enzyme pathways in platelets were presumably damaged.

Smith and Willis (1971) revolutionised the knowledge of the mechanism of action of acetylsalicylic acid on platelets by the demonstration of its effects on prostaglandins. Willis and Kuhn (1973) incubated platelet arachidonic acid with cyclo-oxygenase and demonstrated an unstable factor which induced platelet aggregation. This substance was identified as prostaglanding GH_2, an intermediary /

intermediary of the cyclo-oxygenase reaction (Hamberg and Samuelsson, 1973; Hamberg, Svensson, Wakoliayashi and Samuelsson, 1974; Hamberg, Svensson and Samuelsson, 1975). Platelet prostaglandins are further transformed to an unstable compound, thromboxane A_2, by an enzyme designated thromboxane synthetase which is of great potency as an inducer of platelet aggregation (Needleman, Moncada, Bunting, Vane, Hamberg and Samuelsson, 1976). The anti-aggregatory effect of aspirin was shown to be due to acetylation of the active site of cyclo-oxygenase (Morse, 1977; Raz, Isaakson, Minkes and Needleman, 1977).

Studies on microsomal extracts from blood vessels revealed that prostaglandins are transformed into an unstable vasodilator substance with potent anti-aggregatory activity (Moncada, Gryglewski, Bunting and Vane, 1976). This compound, prostacyclin, is derived from arachidonic acid metabolism in vascular tissue (Gryglewski, Bunting, Moncada, Flower and Vane, 1976) and its generation is also inhibited by acetylation of cyclo-oxygenase (Vane, 1971).

Changes in platelet function and gastrointestinal bleeding following repeated administration of aspirin in rats and dogs were studied by Mills, Lane, Otton, Cook and Philp (1979). The maximum platelet aggregation responses to adenosine diphosphate were found on the third day, which coincided with gastrointestinal bleeding. Baumgartner (1979) concluded that low citrate concentrations in the rabbit inhibit, and strongly enhance a possible inhibitory effect of acetylsalicylic acid on thrombus growth.

The bleeding time was significantly prolonged from a mean of 3.5 to 6.5 minutes following the ingestion of 600 mg aspirin (Stuart et al. 1979), but a combination of 180 mg aspirin and 50 mg /

50 mg dipyridamole 3 times daily had no such effect (Rajah, Penny, Crow, Pepper and Watson, 1979).

The effects of aspirin and dipyridamole on platelet function in divers were reported by Philp, Anderson, Fields, McIntyre, Francy and Briner (1979). A reduction in circulating platelet count was observed in all divers except in the groups who received aspirin only, but platelet survival was shortened in all. The effects of aspirin and aspirin lysinate on platelet function in smokers and non-smokers were also investigated (Morgan, Duchosal, Rogg and Miescher, 1980). Bleeding time and platelet adhesiveness were similar for both groups and inhibition of platelet aggregation was slightly less in the smokers than in the non-smokers.

Differential inhibition of prostacyclin production and platelet aggregation by aspirin was studied by Masotti et al. (1979). They concluded that a dose of 3.5 mg/kg of aspirin is most likely to produce a consistent inhibition of platelet aggregation with relatively less inhibition of prostacyclin production. This was not confirmed by others (Pareti et al., 1980; O'Brien, 1980; Hoogendijk and Ten Cate, 1980; Huijgen et al., 1980). However, Ellis, Wright, Jones, Richardson and Ellis (1980) reported that one-quarter of an aspirin tablet which inhibits a major portion of platelet cyclo-oxygenase may not inhibit vascular cyclo-oxygenase and may be more efficacious as an anti-thrombotic agent in man. Pacciorette and Elock (1980)studied the effects of aspirin on platelet aggregation as a function of dosage and time, and concluded that the inhibition by a single oral dose of aspirin ($\geq$ 81 mg) may be expected to persist for the life of the platelets affected.

Among four antiplatelet drugs studied for the prevention of stroke, /

stroke, aspirin was the only drug effective in reducing the incidence of stroke and death in patients with transient cerebral ischaemia (Hirsh, 1981).

The effects of acetylsalicylic acid in overdosage on platelet function have not been reported. The present study was carried out to investigate the effect of aspirin overdosage on platelet function and haemostasis.

(b) Methods

Ten healthy volunteers (7 males and 3 females) aged 24-51 years weighing 55-82 kg were studied with informed consent. They had not taken any drugs for at least 2 weeks before the study.

On the first day, 30 ml of venous blood was taken; 2 ml was placed in a tube containing soya bean thrombin (Wellcome Reagents Ltd.) for fibrin degradation products assay, 10 ml in a universal tube containing 31.3 mg (0.016M) sodium citrate for the measurement of fibrinogen and plasminogen, and 18 ml in another universal tube containing 2 ml of 3.8% sodium citrate for platelet aggregation studies. Hess's test was performed (Dacie and Lewis, 1975) and the bleeding time measured (Ivy, Nelson and Bucher, 1940). Fibrin degradation products were estimated using latex particles coated with antibody to human fibrinogen fragments D and E (Wellcome Laboratories, Thrombo-Wellcotest, 1979). Fibrinogen was measured by the method of Ellis and Stransky (1961), and plasminogen by the method Alkjaersig, Fletcher and Sherry (1959).

Platelet aggregation was determined with a six channel aggregometer (Malin Electronics, Model 6C AC6) using three twin channel recorders (Mitsui, Limited, Model DBE2). The blood sample was centrifuged /

centrifuged immediately at 250 x g for 10 minutes, and the platelet rich plasma removed. The remaining sample was further centrifuged at 2000 x g for 15 minutes to obtain platelet-poor plasma. Aliquots of the platelet-rich plasma (0.6 ml) were challenged with adenosine diphosphate and adrenalin (2 and 2.5 μM) in the final suspension, and aggregation was followed by measuring the increase in transmission of light as clumping proceeded. The platelet-poor plasma was used to zero the recorder for the respective platelet-rich plasma. Aggregation was followed for 6 minutes and the results expressed as percent transmission obtained at each minute interval. All samples were challenged between 55 and 65 minutes after venepuncture.

On the second day, each subject took 20 mg/kg aspirin dissolved in 200 ml of water following an overnight fast. Food, fluids and tobacco were withheld for 2.5 hours. At 2 and 24 hours after ingestion, the above tests were repeated.

The above tests together with platelet counts and measurement of the prothrombin time ratio were performed (Quick, 1942) in 23 patients (10 males and 13 females) aged 16-59 years, weighing 43-86 kg with mild to severe aspirin poisoning. The patients received different treatment; 7 controls, 7 forced alkaline diuresis, 3 forced diuresis, 3 alkali alone and 3 forced alkaline diuresis with frusemide (Chapter 2, Section IV).

Paired and independent two-tailed Student t-tests were used for comparisons, taking $p < 0.05$ as the minimum level of statistical significance.

(c) Results

Hess's test and bleeding time /

Hess's test and bleeding time

The Hess's test was negative (less than 10 petechiae) in all healthy volunteers and overdose patients.

The effects of aspirin on bleeding time in the normal subjects and in the overdose patients are illustrated in Table 6.6. The bleeding time increased significantly in the normal subjects from a mean of 1.77 minutes before taking the aspirin to 2.72 minutes at 2 hours ($p < 0.0025$), and 2.27 minutes at 24 hours ($p < 0.05$) after ingestion. The patients with aspirin poisoning had a wider range of bleeding time than the healthy subjects. There were no statistically significant differences between the groups of patients. The overall mean was 3.65 minutes, which was significantly higher than the controls ($p < 0.001$) (Fig. 6.2.). There was a significant difference between the bleeding time in the overdose patients and the healthy subjects 24 hours (but not 2 hours) after ingestion ($p < 0.05$). The bleeding times obtained in both the healthy volunteers and overdose patients were all within the accepted normal range (1 - 7 minutes) for the method used.

Plasminogen, fibrinogen and fibrinogen degradation products

The plasma fibrinogen and plasminogen concentrations are given in Table 6.7. Aspirin produced no significant changes in the plasma fibrinogen and plasminogen concentrations in the healthy volunteers. The mean plasminogen concentration was normal in the patients with aspirin poisoning, but plasma fibrinogen concentrations were abnormally low in 6 patients although two of the controls had low concentrations (Fig. 6.2.).

The /

TABLE 6.6. BLEEDING TIMES IN 23 ASPIRIN OVERDOSE PATIENTS AND 10 NORMAL VOLUNTEERS BEFORE, 2 HOURS AND 24 HOURS FOLLOWING ORAL ASPIRIN (20 mg/kg)

	BLEEDING TIME (minutes)			
	Control subjects			Overdose patients
	Before	+ 2 hours	+ 24 hours	
Range	1.10 - 2.75	2.05 - 3.92	1.45 - 2.95	1.58 - 4.50
Mean	1.77	2.72	2.27	3.65
*Significance (p)	-	< 0.0025	< 0.05	< 0.001

Normal range 1 - 7 minutes.

* Compared with normal volunteers before aspirin.

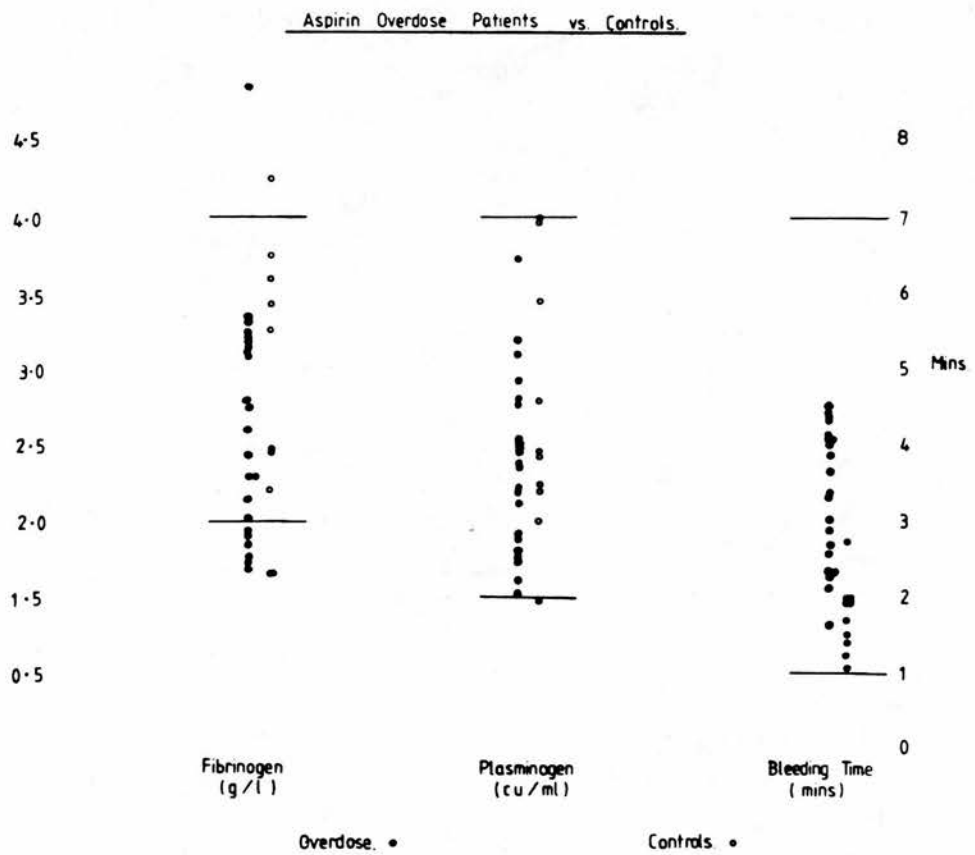

Figure 6.2. Plasma concentrations of fibrinogen and plasminogen and bleeding time in 23 patients with mild to severe aspirin poisoning (●) and 10 healthy subjects (o) who had not taken any drug for 2 weeks before the study.

TABLE 6.7. PLASMA FIBRINOGEN & PLASMINOGEN CONCENTRATIONS IN 23 ASPIRIN OVERDOSE PATIENTS AND 10 NORMAL VOLUNTEERS, BEFORE, 2 HOURS AND 24 HOURS FOLLOWING ORAL ASPIRIN (20 mg/kg)

	FIBRINOGEN (g/l)				PLASMINOGEN (units/ml)			
	Control subjects			Overdose patients	Control subjects			Overdose patients
	Before	+ 2 hours	+ 24 hours		Before	+ 2 hours	+ 24 hours	
Range	1.65 - 4.25	1.88 - 3.91	1.63 - 4.69	1.66 - 4.85	1.46 - 4.0	1.56 - 4.04	1.5 - 4.12	1.52 - 3.72
Mean	2.87	2.78	2.67	2.62	2.70	2.69	2.65	2.36
Normal range	2 - 4 g/l				1.5 - 4.0 units/ml			

None of the differences are statistically significant

The concentrations of plasma fibrinogen degradation products was normal (< 10 μg/ml) in all healthy subjects after taking aspirin. Five of the overdose patients (22%) had increased concentrations >(10 μg/ml).

Platelet aggregation

The results of the platelet aggregation studies are shown in Figure 6.3. In both the healthy subjects and patients with aspirin poisoning, the second phase of platelet aggregation was abolished following challenge with adrenaline. The controls illustrate the classical aggregation pattern with the second wave of aggregation following adenosine diphosphate release from the platelets. The first phase of aggregation was impaired in the overdose patients.

Challenge with adenosine diphosphate (Fig. 6.3.) shows disaggregation of platelets following the first phase response in all groups receiving aspirin. There was no marked difference between the healthy subjects and patients. The platelets of the control subjects responded to stimulation with adenosine diphosphate and there was no subsequent disaggregation before aspirin administration.

Platelet count and prothrombin time ratios in patients with aspirin poisoning

The platelet counts in patients with aspirin poisoning ranged from 81 to 340 x 10^9/l (normal range from 150 to 400 x 10^9/l). Only one patient (No. 5) had a platelet count below normal (81 x 10^9/l). This patient had a previous history of polycythemia and the plasma concentrations of fibrinogen degradation products were increased (> 40 μg/ml). However, the mean platelet count (including Patient No. 5) was 208 x 10^9/l.

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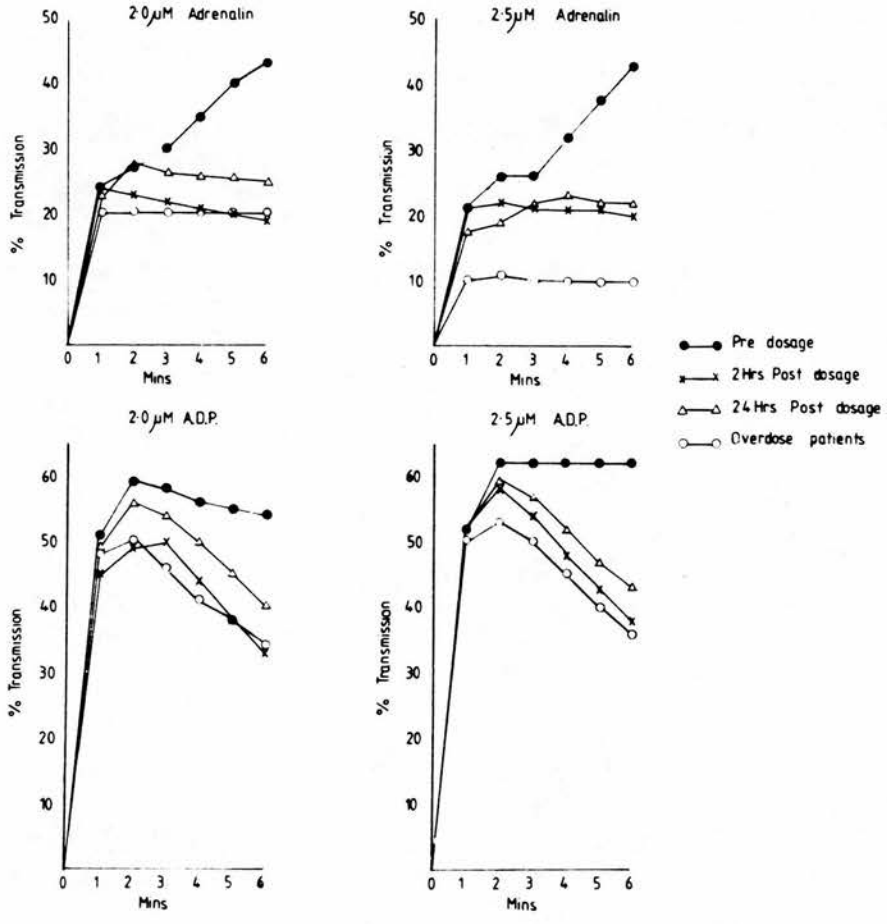

Figure 6.3. Platelet aggregation patterns in 21 patients with mild to severe aspirin poisoning and 10 healthy subjects, before, 2 and 24 hours after a single oral dose of 20 mg/kg aspirin.

The prothrombin time ratio in patients with aspirin overdosage ranged from 1.2 to 1.8 with a mean of 1.4.

(d) Discussion

A significant increase in the bleeding time after therapeutic doses of aspirin has been reported by many investigators (Stuart et al., 1979; Miekle, 1981), but there have been no reports of the effects of toxic doses of aspirin.

Variation in the bleeding time response to low doses of aspirin was noted in the present study, and similar variation was described by Weiss et al (1968). These workers found a significant increase in bleeding time in 6 young males 2 hours after ingestion of 0.3 g aspirin, using the Ivy technique. The slight shortening of the bleeding time at 24 hours compared with 2 hours after ingestion of the aspirin is consistent with the irreversible effect on cyclo-oxygenase activity (Ali et al., 1980), and the entrance of new platelets into the circulation (10% daily). However, the significantly longer bleeding time in the overdose patients relative to that 24 hours after a therapeutic dose indicates a somewhat greater effect following a toxic dose of aspirin (most of the samples from the overdose patients were obtained after 24 hours).

Interpretation of the increased concentrations of fibrinogen degradation products in 5 patients is uncertain, but disseminated intravascular coagulation has been described in salicylate intoxication (Sbarbaro and Bennett, (1977).

The results of the platelet aggregation studies in the healthy volunteers are in good agreement with previous reports (O'Brien, 1968; Evans et al., 1968; Morse, 1977; Rajah et al., 1979; Mills et al., /

al., 1979; Miekle et al., 1981). Following aspirin administration adenosine diphosphate challenge causes disaggregation of platelets after the initial wave of aggregation. This test is relatively crude, but there were no obvious differences in the behaviour of platelets following therapeutic doses and overdosage of aspirin. Adrenaline challenge showed a similar pattern with absence of the second phase of aggregation. The increased prothrombin time ratio in the poisoned patients probably reflects the well-known inhibitory effect of salicylate on the hepatic synthesis of clotting factors (Coldsweig, Kapusta and Schwartz, 1976). In view of these findings, it can be concluded that aspirin in therapeutic doses and overdosage have similar adverse effects on haemostasis.

(e) Summary and conclusions

Haemostasis was compared in 23 patients with aspirin poisoning and in 10 healthy volunteers taking a single oral dose of 20 mg/kg.

The Hess's test was normal in all overdose patients and healthy volunteers.

The bleeding time was significantly increased in the normal subjects from a mean of 1.77 minutes before aspirin to 2.72 minutes at 2 hours ($p < 0.0025$) and 2.27 minutes at 24 hours ($p < 0.05$) after ingestion. The mean bleeding time in the overdose patients was prolonged even further to 3.65 minutes ($p < 0.001$). All values were within the normal range (1 - 7 minutes).

Plasma fibrinogen and plasminogen concentrations were normal in both groups. Fibrinogen degradation products were abnormally increased (> 10 µg/ml) in 5 poisoned patients.

The /

The second phase of platelet aggregation following challenge by adenosine diphosphate and adrenalin was abolished in both the normal subjects and the overdose patients. There were no marked differences between the groups.

The platelet count was normal in all overdose patients except one who had a history of polycythemia.

The mean prothrombin time ratio was increased in the patients with aspirin overdosage and varied from 1.2 to 1.8 (mean 1.4).

SECTION VII

GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

SECTION VII

Chapter 1.

GENERAL DISCUSSION

(a) Acetylsalicylic acid absorption

Acetylsalicylic acid was absorbed rapidly when given in solution (20 mg/kg) to healthy subjects with the mean peak plasma concentration occurring at 30 minutes. This is in agreement with previous reports (Levy, 1961; Leonard, 1963; Rowland et al., 1972). Absorption appeared to be faster when it was given one hour after sodium bicarbonate and the mean peak plasma concentration occurred at 15 minutes. Similar enhancement of aspirin absorption by sodium bicarbonate has been noted by Leonard (1963) and Cook and Hunt (1970).

The absorption of acetylsalicylic acid following overdosage has not been investigated previously, although some investigators have commented on apparent delay in absorption as judged by the recovery of aspirin tablets remaining in the stomach at gastric aspiration and lavage (Matthew et al., 1966; Matthew, 1970; Springer, 1980). Furthermore, delayed peak plasma salicylate concentrations in aspirin poisoning have been reported (Beveridge et al., 1964; Ferguson and Boutros, 1970). Since unchanged acetylsalicylic acid was not measured in these reports, it is impossible to distinguish between delayed absorption and slow hydrolysis of the drug. In the present study very slow and delayed absorption was observed in some patients with aspirin overdosage. Plasma acetylsalicylic acid concentrations continued to increase for as long as 18 hours in one control patient and the unchanged drug was detected in the plasma up to 24 hours after ingestion. This

delayed /

delayed absorption could be due to either slow dissolution of the tablets or slow gastric emptying following overdosage.

(b) Metabolism and distribution

Acetylsalicylic acid was rapidly hydrolysed to salicylic acid and only 1-2% of the dose was excreted unchanged in the urine in the healthy volunteers. This is in general agreement with previous reports (Rowland et al., 1967; Levy, 1978; Levy, 1981). Following overdosage, even less of the total recovered was excreted unchanged (0.5%), but there are no previous reports of the urinary recovery of unchanged acetylsalicylic acid for comparison.

Salicylic acid is the major metabolite of acetylsalicylic acid. It is eliminated by renal excretion and by conjugation and oxidative metabolism (Milne, 1962; Terweij-Groen et al., 1980; Levy, 1981). Conjugation with glycine to form salicyluric acid, is the main route of elimination with low therapeutic doses, but this process is saturated when the amount of salicylate in the body exceeds approximately 360 mg (Levy, 1965b). Salicyl phenolic glucuronide (but not salicyl acyl glucuronide) is also saturated at higher salicylic acid concentrations (Levy et al., 1972). The oxidative metabolites (e.g. gentisic acid) and the recently described new metabolite (gentisuric acid) are minor metabolites (Fig. 1) which account for about 1% of the total urinary recovery of salicylate. The ratio of the urinary recovery of salicylic to salicyluric acids is not only related to the dose absorbed, but also to the urine pH.

Albumin is not the only plasma protein to which salicylic acid is bound as was previously believed (Reynolds and Cluff, 1960; Butterman et al. /

al., 1962). The binding of salicylic acid to albumin accounted for only half of the total bound to plasma proteins at certain concentrations and this has not been reported previously. However, the increase in the fraction unbound to total plasma proteins with increasing plasma salicylic acid concentrations has been reported before (Smith et al., 1946; Wosilait, 1976; Ekstrand et al., 1979; Rowland, 1980). However, there have been no detailed studies of salicylate binding at concentrations encountered following aspirin poisoning. The marked increase in free salicylic acid at high plasma concentrations is of major significance in relation to toxicity after overdosage. Only unbound salicylic acid can pass the blood brain barrier, and therefore its concentration in plasma may closely reflect concentrations in the central nervous system and cerebrospinal fluid. In addition, a fall in blood pH will increase brain and decrease plasma concentrations (Reed and Palmisano, 1975).

The concentration-dependent volume of salicylic acid distribution found in the present study confirmed the findings of Levy and Yaffe (1974), and this effect is probably due to the marked dependence of protein binding on salicylate concentration. In fact, the volume of distribution of unbound salicylic acid increased or remained constant as the total plasma concentrations decreased following overdosage. Diflunisal is extensively bound to plasma proteins (Verbeeck et al., 1980) and this probably accounts for its longer plasma half-life following a therapeutic dose.

Although haemodilution during infusion in the overdose patients treated by forced diuresis regimes was confirmed by serial measurements of haematocrit and plasma albumin concentrations, the apparent volume of salicylic acid distribution did not seem to change significantly. This /

This is probably a pharmacokinetic artefact resulting from the use of inappropriate methods for the calculation of volume of distribution, since plasma and extracellular volumes and salicylate clearance were changing rapidly during the infusion.

(c) Urine pH and flow rate

In the healthy volunteers given aspirin and diflunisal, satisfactory and virtually identical changes in urine pH and flow rate were achieved following the administration of sodium bicarbonate. Hanzlik (1926) failed to enhance salicylate elimination with sodium bicarbonate, but this was presumably because insufficient was given to alkalinize the urine.

In the present study with the overdose patients, there was also a satisfactory increase in urine pH and flow rate, although the immediate increase in flow rate was limited by fluid retention unless frusemide was also given. The highest urine pH was obtained with alkali alone and the highest flow rate with forced alkaline diuresis plus frusemide. Since laevulose and even glucose might be degraded to acidic compounds in alkaline solution (Conn and Stumpf, 1972), it is important not to add the bicarbonate until immediately before use. Even so, autoclaved dextrose solutions are very acidic and some of the added sodium bicarbonate is therefore wasted in neutralisation. The use of sodium bicarbonate alone is therefore, simpler and more effective in raising the urine pH.

The significant positive correlation between the urine pH and flow rate, in the forced alkaline diuresis regimes is an obvious artefact (Morgan and Polak, 1971).

(d) /

(d) The effects of changes in urine pH and flow rate on the plasma half-life and total body clearance of salicylic acid

The significantly lower plasma salicylic acid half-life and increased total body clearance with alkaline diuresis in the healthy volunteers is in agreement with previous reports (Smith et al., 1946; Levy and Leonard, 1971; Levy, 1978). These changes are due entirely to the increased renal clearance of salicylic acid in alkaline urine. The mean reduction in plasma salicylate half-life was 34%. However, this was not the case for diflunisal. Although its renal clearance increased significantly with alkaline diuresis, there was no change in the plasma half-life and total body clearance. However, the renal clearance of diflunisal is very low and only 5-7% of the dose was excreted unchanged in the urine.

The shortening of the apparent plasma salicylic acid half-life in the poisoned patients during infusion of 6 litres of fluid (particularly with forced diuresis) must have been due largely to haemodilution. The changes in haematocrit and plasma albumin, total protein, calcium, phosphate, urate, magnesium and salicyluric acid concentrations all point to significant expansion of the plasma and/or extracellular fluid volumes. In addition, the amounts of salicylic acid recovered during the infusion are too small to account for the observed fall in plasma concentrations. For example, in the forced diuresis group, it can be calculated (from the apparent volume of distribution and the urinary recovery) that the mean plasma concentrations would only have fallen by about 7% without haemodilution. In fact, the observed fall was 33%. However, the decrease in plasma salicylic acid half-life during the infusion period in the alkalinisation groups (particularly /

(particularly alkali alone) was also due to enhanced renal excretion as a result of the increase in urine pH. There was no evidence of haemodilution in the alkali alone group and the observed fall in plasma salicylate concentrations during the first 4 hours (35%) corresponded exactly with the amounts recovered in the urine over the same period.

After the infusion was completed, the plasma salicylic acid half-life became longer in all patients except 3 who received alkali alone. Interestingly, in one of these patients the plasma salicylic acid half-life (2.62 hours) was virtually identical to the shortest individual plasma half-life following a therapeutic dose of aspirin in the healthy volunteers with alkaline diuresis (2.61 hours). This is even shorter than the mean plasma salicylate half-life (2.9 hours) reported with a low therapeutic dose (0.25 g aspirin) in which elimination followed first-order kinetics (Levy, 1965b). In fact, in the present study it was not possible to confirm with certainty the elimination of salicylate by first-order kinetics at toxic concentrations as reported by Levy (1965b, 1978). He mentioned that with very high doses of salicylate, elimination appears to be first-order, since the zero-order contribution is too small to be noticeable except during the terminal elimination phase. This is true, particularly when the urine pH is very high (above 8.0), in which case the zero-order contribution may not be obvious at all. With low urine pH as in the control patients, the renal excretion of salicyluric acid exceeded that of salicylic acid.

In the control patients, the total body clearance of salicylic acid tended to increase with time in some patients as the plasma concentration of salicylic acid declined and this could have been due to /

to a change from zero-order to first-order elimination kinetics. This might also account for the much higher total body clearance of salicylic acid in the healthy volunteers than in the control overdose patients.

Of the different treatment regimes used in the patients with aspirin overdosage, administration of alkali alone produced the greatest reduction in plasma salicylic acid concentration, and in this respect it was at least as good and probably better than any of the other treatments. In addition, the decline in plasma salicylic acid concentration was only matched by a corresponding increase in the urinary excretion in the alkali alone group. Forced diuresis was the least effective treatment and the mean plasma salicylate half-life after completion of the infusion was the same as in the control group.

(e) The effects of changes in urine pH and flow rate on salicylate renal clearance and excretion

A significant positive correlation was found between the renal clearance of salicylic acid and urine pH both in the healthy volunteers and the overdose patients. Similar findings have been reported by numerous investigators (Smith et al., 1946; Lawson et al., 1969; Morgan and Polak, 1969). Morgan and Polak (1971) used mannitol to produce diuresis with sodium lactate, acetazolamide and sodium bicarbonate to raise the urine pH. They also found a better correlation between the urine pH and renal clearance of salicylate, when the urine flow rate was held constant. However, the correlation between flow rate and renal clearance of salicylate became less significant when /

when the urine pH was held constant. The significant correlation between the renal clearance of salicylate and the urine flow rate can be attributed in part to the positive correlation between urine pH and flow rate. McPherson et al. (1955) and Morgan and Polak (1971) believed that a relatively small effect of urine flow rate on the renal clearance of salicylate might be masked by the much greater effect of urine pH as was seen in the present study.

Weiner, Washington and Mudge (1959) studied the renal clearance of salicylate in the dog and showed that salicylate enters the tubular lumen from the plasma by glomerular filtration and tubular secretion. In the filtrate it exists in two forms : salicylate ions and unionized salicylic acid, the proportion of each depending on the pH. The tubular epithelium is highly permeable to the latter, but much less permeable to salicylate ions. Therefore, the extent of reabsorption depends on the concentration gradient of unionized salicylic acid between the tubular fluid and peritubular capillaries and this is related to the urine pH. Salicylic acid is a moderately strong organic acid with a pKa of 3.0. An increase in urine pH increases the fraction present in the ionized form, decreases tubular reabsorption and thus enhances the urinary excretion of salicylate.

The ratios of the concentrations of ionized, unionized and total salicylic acid in the tubular fluid and peritubular capillaries can be calculated using the Henderson-Hasselbalch equation. If it is assumed that the blood pH is 7.45 and that there is diffusion equilibrium of the unionized salicylic acid, the urine/blood ratios of the salicylic acid concentrations for urine pH values of 5.0, 6.0, 7.0, 8.0 and 9.0 are 0.0036, 0.036, 0.36, 3.6 and 36 respectively. The calculation for a urine pH of 5.0 and a blood pH of 7.45 with

a /

a pKa value of 3.0 for salicylic acid is as follows :

$$R = \frac{Cu}{Cp} = \frac{1 + 10^{(\text{urine pH-pKa})}}{1 + 10^{(\text{blood pH-pKa})}} = \frac{1 + 10^{2}}{1 + 10^{4.45}} = 0.0036$$

R is the ratio of urine concentration (Cu) to plasma concentration (Cp) of salicylic acid. Similarly, at a urine pH of 8.0 the ratio is 3.6, a thousand-fold increase.

Thus for each unit increase in urine pH, the renal clearance of salicylic acid should increase ten-fold. In practice, a smaller value is found, perhaps because of protein binding or failure to achieve complete distribution equilibrium, especially at high urine flow rates. In the present study, the factor as determined by multiple regression analyses, was 6.3 with alkali alone, 2.1 with the forced alkaline diuresis and even less with the other treatment regimes (Table 4.24.). Morgan and Polak (1971) found an approximately four-fold increase in salicylate clearance for each rise of one unit in urine pH, irrespective of whether alkaline diuresis was induced with mannitol and sodium lactate or acetazolamide plus sodium bicarbonate.

Although multiple regression analysis indicated that urine flow rate had a negative effect on the ratio of urine to plasma concentrations of salicylic acid, this cannot be the case biologically, since forced diuresis gave a significantly higher renal clearance of salicylic acid than in the controls.

The higher renal clearance of unbound salicylic acid (> creatinine clearance) indicates active tubular secretion as well as glomerular filtration and tubular reabsorption (Milne et al., 1958; Schachter and Manis, 1958; Bedford et al., 1965). Since the renal clearance of /

of unbound salicylic acid was measured only in 5 patients, insufficient data were available for multiple regression analyses of the effect of changes in urine pH and flow rate.

The addition of frusemide to the forced alkaline diuresis regime, had no obvious effect on the urine pH and although it induced a rapid diuresis, it failed to further enhance salicylate elimination. Similar findings were reported by Berg (1977b). Thus, there is no indication for the use of frusemide (or presumably, other diuretics) unless fluid retention is a cause for concern.

Alkaline diuresis produced a significant increase in the renal clearance of salicyluric acid in the healthy volunteers. However, the explanation is unlikely to be pH-dependent tubular reabsorption of salicyluric acid. There was no such correlation between urine pH and salicylurate clearance in the patients with aspirin poisoning, and under these conditions, conjugation of salicylic acid with glycine was fully saturated throughout the period of study, confirming the observations of Levy (1965a, 1965b) and Levy et al. (1969).

The ratio of the urinary recovery of salicylic acid to salicyluric acid increased significantly with alkalinisation of urine both in the healthy volunteers and overdose patients. The ratio was much greater in the overdose patients reflecting saturation of glycine conjugation of salicylic acid in the presence of much larger amounts of salicylic acid (Levy, 1965b, 1978, 1981). Indeed, the urinary recovery of salicyluric acid was essentially the same in all overdose patients, irrespective of the treatment regimes.

Administration of alkali alone in the poisoned patients produced the highest renal clearance and the greatest urinary recovery of salicylic acid. Of the different regimes used, it was the most effective in enhancing renal salicylate elimination following overdosage.

(f) /

(f) The effects of different treatment regimes on clinical and biochemical abnormalities

The effects of the different regimes of fluid and alkali on the clinical manifestations of aspirin poisoning corresponded well with their different effects on the elimination of salicylic acid. Although the manifestations of salicylate toxicity were similar in all the treatment groups during the first 4 hours after admission, the proportion of patients with salicylism 12 hours after admission was lowest in the alkali alone group. Thus, more rapid recovery was obtained with this method of treatment. Other investigators have been preoccupied with salicylic acid kinetics, and no information is available concerning the relative efficacy of different treatments on the clinical features of acetylsalicylic acid poisoning.

The pH-partition theory can be applied to the distribution of salicylate between blood and brain. Elevation of blood pH will tend to reduce the concentrations in the brain and the administration of sodium bicarbonate (alkali alone) has been used for the treatment of salicylate poisoning in children (Temple, 1978; Done, 1978).

The positive fluid balance and weight gain (both at the end of infusion and 12 hours later) was much less with alkali alone than with forced diuresis and forced alkaline diuresis (but not forced alkaline diuresis with frusemide). This is in good agreement with previous reports (Lawson et al., 1969; Savage et al., 1969; Temple et al., 1976; Berg, 1977b) although weight gain (perhaps a more reliable index of fluid retention than fluid balance) was not mentioned. All the changes in haematological and biochemical measurements were consistent with significant fluid retention and haemodilution during infusion in the forced diuresis, but not alkali alone groups.

Fluid /

Fluid retention caused by attempts to induce a diuresis rarely seems to cause serious problems in patients with salicylate poisoning. However, forced diuresis is not without risk and can cause serious problems in the elderly and in patients with cardiac and renal disease (Prescott, 1974). Furthermore, serious and sometimes fatal pulmonary and cerebral oedema has often been reported in patients with aspirin poisoning who were subjected to treatment by diuresis (Proudfoot and Brown, 1969; Ferguson and Boutros, 1970; Whitehall, 1973; Tweedale, 1974; Davis and Burch, 1974; Broderick et al., 1976; Heffner et al., 1979). It is clearly dangerous and unnecessary to use a forced diuresis regime when better results can be obtained simply and more safely by administration of alkali alone.

Different amounts of sodium, potassium and chloride were administered in the different treatment regimes, but the plasma sodium and potassion concentrations did not change significantly and there were no significant differences between the groups.

Hypocalcaemia occurred in all groups of patients, but there were no obvious clinical symptoms or signs and the plasma calcium concentrations eventually returned to normal spontaneously. The most marked falls occurred with forced alkaline diuresis, forced alkaline diuresis plus frusemide and forced diuresis while the least effect was observed with the control and alkali alone groups.

The fall in plasma phosphate concentrations was greatest with the diuresis regime , possibly because of the additional effects of dextrose itself (Guillou et al., 1976), and least with alkali alone. Aspirin overdosage itself produces a fall in plasma phosphate concentrations, since similar but less marked changes occurred in the control group. The explanation is unknown and this effect has not been reported previously.

As /

As expected the mean plasma osmolality on admission was above the normal range in all groups and the subsequent fall was more marked with the diuresis regimes. Other investigators have reported similar findings (Morgan et al., 1968; Berg, 1977b).

The plasma total CO_2 concentration on admission was abnormally low in all groups of patients, but there was rapid improvement with the alkalinisation regimes particularly alkali alone. However, forced diuresis had the opposite effect. The low plasma CO_2 concentrations presumably reflect respiratory alkalosis, although in some patients there may also have been a contribution from metabolic acidosis.

(g) Urinary sodium excretion and renal function

The low urinary excretion of sodium in the patients with aspirin poisoning is consistent with its well known inhibitory effects on renal medullary prostaglandin synthesis (Kimberly et al., 1979; Plotz and Kimberly, 1981). The acute effects of therapeutic doses of aspirin on renal function are thought to be reversible, but other renal homeostatic mechanisms such as induction of antidiuretic hormone may also be involved (Plotz and Kimberly, 1981). Inhibition of prostaglandin E synthesis has been proposed as the mechanism of acute impairment of renal function observed in patients given aspirin (Kimberly et al., 1979; Moncada et al., 1980). However, therapeutic doses of aspirin also produce acute proximal tubular injury (Prescott, 1980). Salicylic acid is as active as aspirin in inhibiting prostaglandin synthesis while in vitro it is much less active (Smith et al., 1979). This suggests that salicylic acid undergoes metabolic activation and in this context it should be noted that gentisic acid is a much /

much more potent inhibitor than salicylic acid (Shen, 1979). Salicyluric acid is apparently inactive, while 4-amino-salicylic acid has the same activity as aspirin (Flower, 1974). These differences must be interpreted with caution however, because of marked species and organ differences in susceptibility to inhibition of prostaglandin synthesis by different compounds..

Prostaglandin E dilates renal medullary arterioles and increases blood flow to the inner cortex at the expense of the outer cortex, while prostaglandin $F_{2\alpha}$ and thromboxane A_2 reduce renal blood flow. Prostaglandins inhibit the effects of antidiuretic hormone on the collecting ducts and also inhibit the reabsorption of sodium from the renal tubules and collecting ducts (Plotz and Kimberly, 1981). The inhibition of synthesis of renal prostaglandins by aspirin probably accounts for the fluid retention and reduced sodium excretion observed following overdosage and these effects are probably due to reduced renal blood flow and glomerular filtration rate (Berg, 1977a, 1977b; Smith et al., 1979; Kimberly et al., 1979). However, these actions alone are unlikely to explain the low creatinine clearance which was observed in half of the patients with aspirin poisoning. Acute tubular necrosis and renal failure are recognised complications of salicylate poisoning (Krasnoff and Bernstein, 1947) and even in therapeutic doses, aspirin produces acute tubular damage in man as indicated by a striking increase in the urinary excretion of renal tubular cells (Scott et al., 1963; Prescott, 1965) The fluid and sodium retention and markedly reduced creatinine clearance in some patients with aspirin poisoning is probably due to a combination of medullary ischaemia (following inhibition of synthesis of prostaglandin E) and proximal tubular necrosis.

(h) /

(h) Haemostasis

There were surprisingly few clinically significant abnormalities of haemostasis following aspirin overdosage. Although the mean bleeding time was significantly higher in the patients than in the healthy volunteers given a dose of 20 mg/kg, the values were still within the normal range (Ivy et al., 1940). Fibrinogen degradation products were raised in 5 patients, suggesting mild disseminated intravascular coagulation, a complication which has been observed previously (Sbarbaro and Bennett, 1977).

The inhibition of platelet aggregation was no different from that observed following therapeutic doses of aspirin (Mills et al., 1979; Miekle et al., 1980). Finally, the mild prolongation of the prothrombin time was of no clinical significance. This effect has often been described following chronic high dose salicylate therapy (Goldsweig et al., 1976). There is clearly no indication for the routine use of vitamin K in aspirin poisoning as was recommended in one recent report (Vale and Meredith, 1980).

SECTION VII

Chapter 2.

SUMMARY AND CONCLUSIONS

(a) Studies with aspirin and diflunisal in healthy volunteers

1. The absorption, metabolism and renal excretion of acetylsalicylic acid was studied in 6 healthy male volunteers following a single oral dose of 20 mg/kg aspirin in solution. The study was then repeated under conditions of alkaline diuresis induced by oral administration of sodium bicarbonate (3 g 4 times daily).

2. Aspirin was rapidly absorbed with peak plasma acetylsalicylic acid concentrations of 17 μg/ml and 15 μg/ml at 15 and 30 minutes with and without sodium bicarbonate, respectively. Acetylsalicylic acid was rapidly hydrolysed to salicylic acid and only 1-2% of the dose was excreted unchanged in the urine.

3. The mean plasma salicylic acid half-life was 3.56 ± 0.89 and 5.66 ± 1.48 hr with and without alkaline diuresis respectively ($p < 0.05$). There was a corresponding increase in the total body clearance of salicylic acid and this was due entirely to the pH-dependent increase in renal clearance of salicylic acid. There was a highly significant correlation between the renal clearance of salicylic acid (corrected for flow rate) and urine pH.

4. The plasma concentrations of salicyluric acid were very low (< 10 μg/ml) and the renal clearance very high (567 ± 84 and 443 ± 117 ml/min with and without alkaline diuresis, respectively).

5. The pattern of urinary excretion of acetylsalicylic acid and its metabolites depends on the urine pH. In the control study, 1.2% of the dose was excreted as acetylsalicylic acid, 8.3% as salicylic acid /

acid and 62% as salicyluric acid, whereas the corresponding values with alkaline diuresis were 2%, 38% and 42.3% respectively. The mean ratio of the urinary recovery of salicylic to salicyluric acid was 0.13 in the control study and 0.90 with alkaline diuresis.

6. Alkalinisation of the urine significantly enhanced the urinary excretion of salicylic acid and reduced the plasma salicylic acid half-life.

7. Similar studies were carried out in 6 healthy volunteers with diflunisal (difluoro-phenyl salicylic acid). This compound is extensively bound to plasma proteins and largely metabolised by glucuronide conjugation.

8. Administration of sodium bicarbonate significantly increased the renal clearance and excretion of diflunisal, but had no effect on the plasma half-life and area under the plasma concentration-time curves. There was no significant correlation between the renal clearance of diflunisal and urine pH or flow rate, but the renal clearance increased with time and declining plasma concentrations of the drug.

(b) Studies in patients with aspirin overdosage

1. Serial plasma and urine concentrations of acetylsalicylic, salicylic and salicyluric acids were measured (using high performance liquid chromatography) in 50 patients with mild to severe aspirin poisoning. The patients received either oral fluids only (controls) or one of four intravenous regimes of fluid and alkali (over 3 hr) to establish the relative importance of urine pH and flow rate on salicylate elimination. Sixteen patients were treated with forced alkaline diuresis (6 L of isotonic dextrose and saline solution containing /

containing 18.9 g of sodium bicarbonate and 9 g of potassium chloride), 6 with forced diuresis (as above, but without the sodium bicarbonate), 6 with forced alkaline diuresis plus frusemide (the same regime as forced alkaline diuresis plus 80 mg of frusemide intravenously) and 6 with alkali alone (1.5 litres of 1.26% sodium bicarbonate containing 4.5 gm of potassium chloride).

Serial measurements were made of fluid balance, weight changes, haematocrit and plasma and urinary osmolality together with concentrations of electrolytes and creatinine. In addition, serial measurements were made of the plasma concentrations of urea, albumin total protein, calcium, magnesium, phosphate and urate, together with liver function tests. The effects of aspirin on haemostasis were compared in the overdose patients and healthy volunteers given a single oral dose of 20 mg/kg.

The clinical manifestations of salicylate poisoning were assessed on admission and 4 and 12 hours later.

The binding of salicylic acid to plasma proteins and albumin was studied in vitro and in the patients with aspirin overdosage using an ultrafiltration technique.

2. The absorption of acetylsalicylic acid was delayed despite gastric aspiration and lavage in some patients as judged by the persistence of acetylsalicylic acid in the plasma and in some cases, increasing plasma concentrations of salicylic acid. Presumably, there was slow dissolution of tablets and/or delayed gastric emptying.

3. In the 16 control patients the mean salicylic acid plasma half-life was 30 ± 9 hours. Glycine conjugation of salicylic acid was saturated as shown by low and relatively constant plasma concentrations of salicyluric acid (1-10 μg/ml). The mean total body clearance of salicylic acid was only 5.4 ± 1.9 ml/min compared with 23.2 ± 5.1 ml/min /

ml/min in the healthy volunteers. The mean renal clearance of salicylic acid (1.4 ± 1.4 ml/min) was also less than that of the healthy volunteers (2.2 ± 1.4 ml/min). The mean renal clearance of salicyluric acid was similar (493 ± 317 ml/min).

The mean urinary recovery of salicylic acid over the first 16 hours was very low (376 ± 320 mg) while 1116 ± 549 mg of salicyluric acid was excreted over that period. The mean ratio of urinary recovery of salicylic to salicyluric acid was 0.48.

4. In the patients given different regimes of fluid and alkali, there was a rapid fall in plasma salicylic acid concentrations during the infusions. The mean plasma half-life values during the first 4 hours were 8.5, 6.6, 6.4 and 5.1 hours in the forced diuresis, forced alkaline diuresis plus frusemide, forced alkaline diuresis and alkali alone groups, respectively. This fall in plasma salicylic acid concentrations could not be accounted for (except in the patients given alkali alone) by enhanced urinary salicylate excretion. The amounts of unchanged salicylic acid recovered in the urine during the first 4 hours were 162, 437, 2171, 1551 and 2435 mg in the control, forced diuresis, forced alkaline diuresis plus frusemide, forced alkaline diuresis and alkali alone groups, respectively.

5. The rapid fall in plasma salicylic acid concentrations during the infusion was due in part to haemodilution as shown by fluid retention, weight gain and reduction in the haematocrit, and plasma concentrations of albumin, calcium, magnesium, phosphate, urate and salicyluric acid. The mean plasma osmolality on admission was abnormally high in all groups. The mean values were within the normal range 4 hours after admission except in the forced diuresis group in whom the mean plasma osmolality at 4 and 12 hours was 275 and 278 mmol/kg respectively. Fluid retention was minor in the control patients /

patients and in those given alkali alone and forced alkaline diuresis with frusemide, and most marked in those given forced diuresis and forced alkaline diuresis. There may also have been changes in the distribution of salicylic acid, although it was not possible to show that the apparent volume of distribution was increased by treatment.

Changes in the apparent plasma half-life of salicylic acid during forced alkaline diuresis are often used as an index of the efficacy of removal of the drug following overdosage. This is clearly inappropriate. In the patients given forced diuresis the fall was due almost entirely to haemodilution while in those given alkali alone the fall corresponded exactly to that predicted from the increased urinary excretion of salicylic acid.

6. During the 12 hours after the infusion was completed, there were marked differences in the plasma salicylic acid half-lives of the different treatment groups. The mean plasma half-life in the patients given forced diuresis was 30.6 hours - virtually the same as in the control patients (30.1 hours). The mean urine flow rate in the former group was 5.8 ml/min compared with 1.4 ml/min in the controls. Diuresis alone was clearly ineffective in enhancing the elimination of salicylic acid.

The mean plasma salicylic acid half-lives over the period of 4-16 hours in the patients given forced alkaline diuresis, forced alkaline diuresis plus frusemide and alkali alone were 11.6, 14.1 and 9.1 hours, respectively. Alkali alone appeared to be the most effective treatment.

7. The mean urinary recovery of unchanged salicylic acid during the first 16 hours was 1529, 3432, 3460 and 3871 mg with forced diuresis, forced /

forced alkaline diuresis plus frusemide, forced alkaline diuresis and alkali alone, respectively. Thus the urinary excretion of salicylic acid was inversely related to the plasma half-life in the different treatment groups. The urinary recovery of salicylic acid was greatest in the alkali alone group.

8. There were marked changes in the pattern of urinary excretion of acetylsalicylic acid metabolites in the different groups. The excretion of salicyluric acid was similar in all groups, reflecting saturation of glycine conjugation of salicylic acid. On the other hand, the proportion of the total excreted as salicylic acid depended on the urine pH. The ratios of the urinary recovery of salicylic to salicyluric acid over the first 16 hours after admission were 1.93, 3.23, 4.54 and 4.40 with forced diuresis, forced alkaline diuresis plus frusemide; forced alkaline diuresis and alkali alone respectively.

9. The mean renal clearances of salicylic acid over the first 16 hours were 4.4, 13, 18 and 24 ml/min with the forced diuresis, forced alkaline diuresis plus frusemide, forced alkaline diuresis and alkali alone groups, respectively.

10. The renal clearance of salicylic acid was highly dependent on urine pH and there were highly significant correlations between the renal clearance, urine pH and plasma half-life. The highest mean urine pH was obtained with alkali alone (8.1) and the lowest (6.5) with the forced diuresis regime.

11. The renal clearance of acetylsalicylic acid, but not salicyluric acid was correlated with urine pH. The mean renal clearance of salicyluric acid was 454 ml/min and there were no significant differences between the control and treatment groups.

12. /

12. Multiple regression analyses gave regression coefficients for urine pH against the ratio of urine to plasma salicylic acid concentration which were 0.53, 0.49, 0.32, 2.06 and 6.26 for the control, forced diuresis, forced alkaline diuresis plus frusemide, forced alkaline diuresis and alkali alone groups, respectively. Thus, for each increase of one unit in urine pH, the ratio of urine to plasma salicylic acid concentrations increased 6.26 fold in the alkali alone group, 2.06 fold in the forced alkaline diuresis and much less in the other three groups. Paradoxically, the regression coefficiants for flow rate were all negative and changes in flow rate had no marked effect on salicylate elimination.

13. The binding of salicylic acid to plasma proteins decreased as the plasma salicylate concentrations increased both in vitro and in vivo. Thus the percentage unbound rose from approximately 20% at a total plasma salicylate concentration of 100 μg/ml to 75% at 600 μg/ml. This finding is of major toxicological significance since the tissue (including central nervous system) salicylate concentration closely reflects the unbound salicylic acid concentration in plasma. The binding of salicylic acid to albumin accounted for about half of the total binding to plasma proteins.

14. Tinnitus was present in 91% of all patients on admission and correlated well with plasma salicylic acid concentrations. Subjective deafness occurred in 63% patients, while sweating, hyperventilation, nausea, vomiting, epigastric pain/tenderness and flushing occurred in 33% - 56% of the patients.

The most rapid symptomatic improvement was observed with the alkali alone regime.

15. /

15. Although different amounts of sodium, potassium and chloride were given in the different treatment regimes, there were no significant differences in the plasma sodium and potassium concentrations between the groups and no significant changes during the infusion in any of the groups.

16. The urinary sodium excretion was very low in the control patients (50 mmol/24 hr), but not in the treated groups, presumably because of the sodium administered during the infusion. Similarly, the urinary potassium excretion was normal in the control patients, but increased in the treated groups.

17. The creatinine clearance was less than 100 ml/min in 15 of the 30 patients in whom it was measured. The lowest value was 18 ml/min in a 21 year old female and in 9 (30%) the creatinine clearance was 75 ml/min or less. The established nephrotoxicity of salicylate together with the reduction in medullary blood produced by inhibition of prostaglandin E synthesis probably account for the marked fluid retention which occurs when attempts are made to force a diuresis in patients with aspirin poisoning.

18. The adverse effects of acetylsalicylic acid on haemostasis were similar in the healthy volunteers given a dose of 20 mg/kg and in the overdose patients. However, the bleeding time was increased to a greater extent following overdosage and in 5 patients there was evidence of disseminated intravascular coagulation.

19. Of the different regimes of fluid and alkali studied for the treatment of aspirin poisoning, administration of 1.5 litres of 1.26% sodium bicarbonate alone was the simplest, most effective and probably safest. It produced the greatest fall in plasma salicylate concentrations, the biggest increase in the renal clearance and urinary excretion /

excretion of salicylic acid and the most rapid relief of the symptoms and signs of poisoning. Unlike forced diuresis and forced alkaline diuresis, it did not cause fluid retention and weight gain and there were no significant biochemical disturbances. The addition of frusemide to the standard regime of forced alkaline diuresis did not increase salicylate elimination and forced diuresis was virtually ineffective.

Administration of sodium bicarbonate without excessive fluid appears to be the treatment of choice for aspirin poisoning and further comparative studies are required with larger numbers of patients.

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APPENDIX I

INDIVIDUAL PLASMA CONCENTRATIONS AND URINARY EXCRETION DATA

PLASMA CONCENTRATIONS OF ACETYLSALICYLIC ACID (μg/ml)

Subject	Condition	Hours after ingestion									
		¼	½	¾	1	1½	2	3	5	8	24
MB	Control	15.7	8.0	6.7	3.6	2.2	5.3	N.D.*	N.D.	N.D.	N.D
	with $NaHCO_3$	23.7	13.6	6.8	3.6	1.9	1.9	N.D.	N.D.	N.D.	N.D.
ML	Control	8.1	12.9	11.0	7.6	4.1	1.9	1.8	N.D.	N.D.	N.D.
	with $NaHCO_3$	13.1	18.2	14.4	7.0	4.3	N.D.	N.D.	N.D.	N.D.	N.D.
JC	Control	5.6	6.9	8.5	6.5	5.5	4.5	3.7	3.0	N.D.	N.D.
	with $NaHCO_3$	8.5	10.6	8.2	8.0	4.2	3.8	2.5	2.6	N.D.	N.D.
HR	Control	23.3	31.2	20.2	13.2	3.7	N.D.	N.D.	N.D.	N.D.	N.D.
	with $NaHCO_3$	20.9	14.1	11.7	8.9	3.8	N.D.	N.D.	N.D.	N.D.	N.D.
RA	Control	8.4	22.2	11.6	5.4	2.0	N.D.	N.D.	N.D.	N.D.	N.D.
	with $NcHCO_3$	16.2	15.7	8.7	6.0	5.6	2.8	2.2	2.5	N.D.	N.D.
IK	Control	17.4	10.0	7.6	7.4	2.7	N.D.	N.D.	N.D.	N.D.	N.D.
	WITH $NaHCO_3$	19.8	14.1	11.4	9.4	4.2	2.7	2.7	2.7	N.D.	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC ACID (μg/ml)

Subject	Condition	Hours after ingestion									
		¼	½	¾	1	1½	2	3	5	8	24
MB	Control	34.1	47.5	59.5	58.3	58.9	76.1	81.1	67.7	55.8	6.9
	with $NaHCO_3$	56.7	81.9	94.4	98.3	86.2	96.0	85.5	76.4	42.3	N.D.
ML	Control	12.8	32.8	56.3	60.0	66.3	68.7	67.1	60.7	41.6	N.D.*
	with $NaHCO_3$	20.2	51.8	68.7	83.9	84.8	83.1	63.4	44.5	18.0	N.D.
JC	Control	6.1	17.4	33.0	42.7	46.8	46.2	70.1	57.4	48.1	2.5
	with $NaHCO_3$	17.6	36.1	46.8	56.1	68.0	66.4	60.3	37.8	19.5	N.D.
HR	Control	16.4	29.0	71.5	80.3	75.9	74.0	71.1	50.5	38.8	N.D.
	with $NaHCO_3$	34.9	63.3	67.6	87.2	79.0	78.5	71.8	39.4	25.3	N.D.
RA	Control	11.9	54.6	75.2	77.9	80.0	75.5	66.9	49.7	33.4	3.0
	with $NaHCO_3$	27.6	64.5	67.4	72.0	74.4	70.0	61.4	43.2	20.7	N.D.
IK	Control	33.5	45.0	55.0	65.8	66.3	63.8	56.6	43.9	25.1	N.D.
	with $NaHCO_3$	48.4	55.4	73.1	83.9	76.0	77.1	61.7	37.2	15.8	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLURIC ACID (μg/ml)

Subject	Condition	Hours after ingestion									
		¼	½	¾	1	1½	2	3	5	8	24
MB	Control	0.69	1.3	1.6	1.6	1.8	2.3	2.3	2.3	2.0	0.94
	with $NaHCO_3$	0.71	1.3	1.5	1.7	1.6	2.0	1.9	1.8	1.4	N.D.
ML	Control	N.D.*	1.6	2.1	2.3	2.2	2.6	2.6	2.2	1.9	N.D.
	with $NaHCO_3$	N.D.	1.0	1.5	1.4	1.5	1.6	1.7	1.5	1.1	N.D.
JC	Control	N.D.	0.57	1.1	1.2	1.8	1.5	1.6	1.8	1.3	N.D.
	with $NaHCO_3$	0.56	1.0	1.2	1.5	1.6	1.5	1.8	1.6	1.1	N.D.
HR	Control	N.D.	1.5	1.7	1.8	1.9	2.0	2.3	2.0	1.66	0.76
	with $NaHCO_3$	0.77	0.93	1.4	1.5	1.5	1.7	1.4	1.4	1.3	N.D.
RA	Control	0.38	1.7	2.1	2.6	3.0	3.0	2.9	3.7	3.2	0.42
	with $NaHCO_3$	0.70	1.6	2.0	1.9	2.4	2.2	2.2	1.9	1.7	N.D.
IK	Control	1.4	1.6	1.7	1.9	2.2	2.2	2.2	1.9	1.2	N.D.
	with $NaHCO_3$	1.2	1.4	1.5	1.9	1.7	2.0	2.0	1.8	1.1	N.D.

* not detectable

ASPIRIN STUDY

URINARY EXCRETION DATA

IN HEALTHY VOLUNTEERS

Subject & treatment	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
M.B. Control	0 - 2	44	5.8	12.4	2384	60.2
	2 - 4	43	5.9	24.0	2333	20.3
	4 - 6	110	5.6	5.13	1168	11.1
	6 - 8	130	5.5	5.57	811	N.D.*
	8 - 10	95	5.6	5.22	1012	N.D.
	10-15	265	6.3	47.0	976	N.D.
	15-24	585	6.4	25.5	531	N.D.
M.B. with $NaHCO_3$	0 - 2	60	6.9	237	1162	189
	2 - 4	790	7.3	75.4	156	N.D.
	4 - 6	940	6.9	42.3	105	N.D.
	6 - 8	400	7.5	171	204	N.D.
	8 - 10	305	7.4	148	250	N.D.
	10 - 12	580	7.2	148	68	N.D.
	12 - 24	1495	7.5	133	44	N.D.
M.L. Control	0 - 2	36	5.9	18.6	1969	143
	2 - 4	115	6.0	15.4	1125	12.3
	4 - 6	107	7.1	192	1078	N.D.
	6 - 8	85	6.5	125	1221	N.D.
	8 - 10	290	5.9	18.7	391	N.D.
	10 - 12	205	7.4	89.6	567	N.D.
	12 - 24	600	6.6	28.0	454	N.D.
M.L. with $NaHCO_3$	0 - 2	130	7.9	475	742	169
	2 - 4	1640	7.5	79.7	75.0	N.D.*
	4 - 6	410	7.7	174	267	N.D.
	6 - 8	1236	7.6	40.3	71.2	N.D.
	8 - 10.5	1130	7.8	25.1	66.5	N.D.
	10.5 - 12	1000	7.7	13.5	38.2	N.D.
	12 - 24	2115	7.6	5.26	24.4	N.D.

* not detectable

ASPIRIN STUDY

URINARY EXCRETION DATA

IN HEALTHY VOLUNTEERS

Subject & treatment	Hours after ingestion	Volume (ml)	pH	Urine concentrations (μg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
J.C.	0-2	150	5.8	21.6	474	55.5
Control	2-4	96	5.9	32.2	1011	43.5
	4-6	72	7.1	301	2338	N.D.*
	6-8	135	7.4	301	757	N.D.
	8-10	158	6.2	40.5	502	N.D.
	10-12	310	5.9	19.4	559	N.D.
	12-24	480	6.1	26.2	531	N.D.
J.C.	0-2	270	7.4	192	327	90.2
with	2-4	800	7.3	106	134	N.D.
$NaHCO_3$	4-6	505	7.9	217	224	N.D.
	6-8.5	580	8.0	198	314	N.D.
	8.5-11	117	7.7	156	696	N.D.
	11-12	242	7.4	19.1	149	N.D.
	12-24	2780	7.3	N.D.	27.6	N.D.
H.R.	0-2	57	6.6	348	1569	352
Control	2-4	70	6.4	186	1523	11.6
	4-6	26	8.0	744	814	3.40
	6-8	25	6.7	145	793	N.D.
	8-10	106	7.0	181	756	N.D.
	10-12	96	6.3	49	823	N.D.
	12-24	445	6.1	15.5	442	N.D.
H.R.	0-2	95	7.8	947	889	313
with	2-4	252	7.6	251	395	5.75
$NaHCO_3$	4-6	195	7.8	415	566	N.D.
	6-8	245	7.7	179	303	N.D.
	8-10	1185	7.4	126	50.3	N.D.
	10-12	685	7.4	21.3	79.6	N.D.
	12-24	1620	7.2	5.56	51.3	N.D.

* not detectable

ASPIRIN STUDY

URINARY EXCRETION DATA

IN HEALTHY VOLUNTEERS

Subject & treatment	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
R.A.	0-2	80	5.4	54.2	2186	85.7
Control	2-4	55	5.6	25.3	2333	7.4
	4-6	75	5.2	10.7	2161	N.D. *
	6-8	78	5.3	7.30	1775	N.D.
	8-10.25	135	5.6	4.02	1136	N.D.
	10.25-12	116	6.7	33.4	811	N.D.
	12-24	440	6.0	17.6	610	N.D.
R.A.	0-2	130	8.0	335	719	169
with	2-4	650	7.4	85.5	179	N.D.
$NaHCO_3$	4-6	1860	7.5	46.0	59.2	N.D.
	6-8	1780	7.3	37.6	55.0	N.D.
	8-10	1610	7.4	23.0	54.2	N.D.
	10-12	1260	7.6	20.3	58.0	N.D.
	12-24	835	7.0	11.5	106	N.D.
I.K.	0-2	92	7.8	604	1111	395
Control	2-4	110	7.0	199	1093	5.65
	4-6	100	7.1	315	1061	N.D.
	6-8	184	7.1	182	613	N.D.
	8.10	212	6.8	91.0	382	N.D.
	10-12	560	7.1	28.3	123	N.D.
	12-24	645	6.3	10.8	198	N.D.
I.K.	0-2	285	7.9	387	327	153.4
with	2-4	680	7.4	150	184	N.D.
$NaHCO_3$	4-6	1450	7.6	86.4	99.5	N.D.
	6-8	1480	7.4	30.6	56.3	N.D.
	8-10	470	7.5	53.5	137	N.D.
	10-12	800	7.3	7.90	41.0	N.D.
	12-24	1165	7.5	5.30	42.5	N.D.

* not detectable

PLASMA CONCENTRATIONS OF DIFLUNISAL (μg/ml)

Subject	Hours after ingestion treatment	½	1	2	3	4	8	12	24	36	48	72
M.B.	Control	1.2	2.0	27	104	87	71.2	39.7	20.2	11.2	4.8	0.9
	with $NaHCO_3$	3.1	9.8	60	102	90	51	37.5	30	11.0	8.0	1.9
I.K.	Control	18.5	22	81	105	90	61.5	54.5	29	14	6.0	1.4
	with $NaHCO_3$	9.1	48.6	95.2	111	124	91.4	60.2	34.2	20	10.4	2.3
H.R.	Control	5	15	50	113	100	75	59	35	18.5	8	1.1
	with $NaHCO_3$	15	66.3	103	116	103	75	50	21.7	13	6.1	0.8
B.M.	Control	34	70	116	69.7	93.5	65.5	51.3	30.5	12.8	7.8	2.5
	with $NaHCO_3$	32	68.3	120	104	94.5	66.9	54.2	21.7	11	5.8	0.9
M.K.	Control	4.2	9.6	44.7	139	104	84.1	64.9	29.5	12.8	7.3	1.5
	with $NaHCO_3$	1.4	11.3	113	135	120	76.2	68	47.6	20	8.6	1.1
R.A.	Control	0.01	24.5	54.9	93.1	89.2	71.6	53.9	30.6	19.6	9.0	2.4
	with $NaHCO_3$	4.1	26.9	71.1	78.8	113	78.8	55.8	33.6	21.1	13.3	3.8

URINARY EXCRETION DATA IN HEALTHY VOLUNTEERS

DIFLUNISAL STUDY

Subject	Hours after ingestion	Treatment: Control			Treatment: with NaHCO3		
		Volume (ml)	pH	Diflunisal concentration (µg/ml)	Volume (ml)	pH	Diflunisal concentration (µg/ml)
M.B.	0- 2	50	5.6	6.8	56	8.6	26.3
	2- 4	25	5.9	14.2	160	6.1	7.0
	4- 6	70	5.8	5.9	120	6.6	11.7
	6- 8	150	5.6	4.2	150	7.3	23.1
	8-10	170	5.4	3.0	300	6.6	5.4
	10-12	260	5.8	3.0	690	7.1	3.3
	12-24	450	5.7	7.4	2610	7.4	7.6
	24-36	420	5.6	6.5	2200	7.7	6.4
	36-48	490	6.6	3.6	1720	7.7	8.1
	48-60	540	5.8	3.0	1750	7.6	4.1
	60-72	750	5.6	1.5	790	6.2	2.3
I.K.	0- 2	285	5.9	2.0	290	8.2	5.1
	2- 4	65	5.9	7.7	750	7.8	3.2
	4- 6	65	7.0	16.0	640	7.8	5.1
	6- 8	126	7.4	11.5	600	7.6	4.2
	8-10	112	6.5	3.0	640	7.2	2.7
	10-12	70	6.1	2.0	615	7.9	6.0
	12-24	640	5.7	3.7	730	7.2	12.7
	24-36	860	6.8	3.8	3635	7.9	2.9
	36-48	1340	6.5	1.6	730	7.7	7.3
	48-60	1160	7.0	2.0	1040	7.8	6.3
	60-72	1100	7.3	0.9	810	6.5	1.4
H.R.	0- 2	76	6.2	2.6	45	8.0	31
	2- 4	46	5.9	12.0	30	6.8	22.7
	4- 6	48	5.8	7.4	65	6.5	9.6
	6- 8	170	5.8	3.2	120	7.0	12
	8-10	160	6.5	3.6	70	8.1	18.4
	10-12	96	6.1	5.2	130	8.7	22.7
	12-24	500	5.9	5.2	860	8.1	6.9
	24-36	985	6.7	2.4	1600	8.2	3.8
	36-48	1100	7.3	3.4	1890	7.1	0.53
	48-60	870	6.4	1.5	1470	7.7	0.4
	60-72	630	6.1	1.0	910	6.6	0.2
B.M.	0- 2	70	6.0	5.2	120	8.6	13.1
	2- 4	174	6.5	8.3	178	7.9	17.7
	4- 6	236	7.1	11.2	1150	7.5	2.7
	6- 8	206	6.8	8.7	950	7.5	2.7
	8-10	89	6.0	19.3	290	7.4	10.0
	10-12	65	5.7	17.5	475	7.7	9.2

URINARY EXCRETION DATA IN HEALTHY VOLUNTEERS

DIFLUNISAL STUDY

Subject	Hours after ingestion	Treatment: Control			Treatment: with NaHCO3		
		Volume (ml)	pH	Diflunisal concentration (µg/ml)	Volume (ml)	pH	Diflunisal concentration (µg/ml)
B.M. (Cont'd)	12-24	417	5.8	16.8	1490	7.5	9.8
	24-36	245	5.5	23.9	3805	7.4	2.2
	36-48	385	5.8	7.8	960	7.6	2.4
	48-60	237	5.6	7.4	2040	7.4	1.6
	60-72	495	6.5	3.5	660	6.9	2.2
M.K.	0- 2	65	7.5	7.2	110	8.6	5.2
	2- 4	95	6.9	14.4	60	8.8	91.8
	4- 6	76	6.5	19.8	95	8.2	85.5
	6- 8	38	6.8	16.5	180	7.7	24.1
	8-10	25	6.5	18.2	520	7.7	8.0
	10-12	25	6.1	18.0	1170	7.6	2.8
	12-24	520	6.0	13.8	1540	7.3	8.7
	24-36	1100	7.1	8.1	2130	7.7	8.0
	36-48	800	6.8	5.8	2420	7.5	1.0
	48-60	380	6.0	7.4	1010	7.3	1.4
	60-72	920	7.3	2.7	925	7.1	0.8
R.A.	0- 2	121	7.3	5.4	75	8.2	18.9
	2- 4	70	7.2	30.8	425	7.1	3.9
	4- 6	70	6.2	13.7	1025	7.2	1.9
	6- 8	76	5.7	8.65	1160	7.2	1.8
	8-10	73	5.6	13.9	540	7.1	4.6
	10-12	53	5.5	8.9	1185	7.4	3.3
	12-24	415	5.8	16.3	1245	6.8	7.3
	24-36	750	6.7	6.1	4140	7.6	4.0
	36-48	505	7.2	9.7	1935	6.8	2.8
	48-60	530	6.5	3.5	1120	7.1	2.5
	60-72	990	6.2	2.0	640	6.1	2.3

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

CONTROL

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
1	3	243	3.3	28
	4	290	4.9	25
	5	320	3.8	22
	6	350	3.6	20
	7	390	3.6	N.D.*
	11	355	3.6	N.D.
	15	302	3.6	N.D.
	17	295	3.6	N.D.
	23	238	3.6	N.D.
	35	163	3.3	N.D.
4	6	290	1.9	N.D.
	7.50	250	2.62	N.D.
	15.25	192	2.89	N.D.
	22.50	170	2.90	N.D.
6	2.83	296	1.9	6.3
	3.25	318	1.9	4.6
	3.92	329	2.0	1.8
	4.83	306	1.7	N.D.
	5.83	282	1.8	N.D.
	8	252	1.8	N.D.
	17.66	205	2.4	N.D.
7	4	308	2.5	N.D.
	4.92	314	3.02	N.D.
	5.92	292	4.0	N.D.
	8.42	263	4.1	N.D.
	17.25	197	3.9	N.D.
	21	172	4.0	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

CONTROL

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
8	8.16	363	3.17	N.D.*
	10.75	317	2.75	N.D.
	13.5	303	2.93	N.D.
	22.75	231	2.51	N.D.
	25.83	233	3.23	N.D.
9	8	364	3.44	N.D.
	9	435	2.89	N.D.
	13	299	1.59	N.D.
	24.75	233	3.82	N.D.
	29.58	201	4.27	N.D.
13	2	264	3.0	24.3
	3	277	2.8	8.9
	4.16	272	2.64	4.2
	7.16	272	2.88	3.3
	16.66	227	3.24	N.D.
16	3.5	391	4.47	N.D.
	5.25	327	4.38	N.D.
	6.5	272	2.66	N.D.
	10.75	260	3.36	N.D.
	14.58	226	3.48	N.D.
	34.25	122	3.72	N.D.
34	3.25	328	2.29	5.0
	4.25	373	2.54	N.D.
	5.25	339	2.46	N.D.
	8.25	258	2.02	N.D.
	12.25	233	3.0	N.D.
	14.58	231	3.06	N.D.
	16.93	207	3.17	N.D.
	20.50	177	3.20	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

CONTROL

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
35	3	276	2.44	N.D.*
	3.5	280	3.14	N.D.
	5	254	2.88	N.D.
	10.5	212	3.65	N.D.
	13.5	215	4.24	N.D.
	16	178	4.3	N.D.
	22	157	4.3	N.D.
36	16.42	336	1.85	N.D.
	17.58	316	1.80	N.D.
	21.16	299	1.81	N.D.
	23.83	258	1.87	N.D.
	32.33	235	2.65	N.D.
	35.58	186	2.17	N.D.
43	14	441	4.70	N.D.
	14.66	453	5.06	N.D.
	16	384	3.15	N.D.
	17.25	387	3.73	N.D.
	18.75	385	3.51	N.D.
	22	366	4.63	N.D.
	24	362	4.56	N.D.
	26.5	324	4.59	N.D.
	36.5	235	4.41	N.D.
	48.5	119	3.17	N.D.
	60	14.30	2.21	N.D.
45	12.75	319	1.47	3.5
	13.25	333	1.71	2.96
	13.92	338	1.94	3.14

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

CONTROL

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
45 (Continued)	15.92	346	2.75	2.35
	17.5	338	2.79	2.74
	18.5	323	2.84	2.31
	20.25	321	2.75	2.0
	23.5	316	2.59	2.0
	33.83	237	2.43	N.D.*
	35.66	223	2.19	N.D.
	39.25	221	2.44	N.D.
48	3.5	398	1.18	N.D.
	5.5	368	1.10	N.D.
	8.5	340	1.64	N.D.
	11	340	1.50	N.D.
	13.58	315	1.34	N.D.
	20	280	1.65	N.D.
	29.33	198	1.32	N.D.
	36	127	1.43	N.D.
50	14.75	251	1.22	N.D.
	15.75	260	0.98	N.D.
	16.75	223	1.13	N.D.
	18.75	210	1.31	N.D.
	20.75	209	1.35	N.D.
	24.5	172	0.94	N.D.
	27	142	1.3	N.D.
	35.25	136	1.31	N.D.
	41.25	80	1.11	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

CONTROL

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
52	3.5	334	1.52	N.D*
	5	345	1.60	N.D.
	10.25	279	1.47	N.D.
	13.5	270	1.90	N.D.
	19	235	1.91	N.D.
	25	221	2.02	N.D.
	31.5	140	1.80	N.D.
	41.5	112	1.87	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Plasma concentrations (µg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
2	8.0	467	2.51	N.D.*
	9.0	380	1.90	N.D.
	10.0	288	2.00	N.D.
	11.0	225	2.11	N.D.
	15.0	146	2.30	N.D.
	17.25	101	2.81	N.D.
	21.0	50.7	2.50	N.D.
	35.5	N.D.	N.D.	N.D.
3	4.75	473	2.15	11.3
	5.75	244	1.34	N.D.
	6.75	302	2.08	N.D.
	11.25	257	1.88	N.D.
	23.00	129	1.66	N.D.
	46.33	7.7	0.81	N.D.
5	3.58	451	3.6	8.2
	4.67	467	5.1	N.D.
	5.5	438	4.2	N.D.
	7.5	331	3.3	N.D.
	10	282	2.91	N.D.
	11	262	3.22	N.D.
	12.5	236	3.33	N.D.
	14.5	205	3.91	N.D.
	18.5	152	2.60	N.D.
	26.5	74.3	2.42	N.D.
	38	9.1	1.50	N.D.
10	17.25	556	6.54	7.38
	17.67	571	7.05	5.48
	18.67	513	5.38	N.D.
	19.75	449	4.62	N.D.
	21.17	368	4.27	N.D.
	21.83	364	4.94	N.D.
	24	322	5.13	N.D.
	26.17	291	5.96	N.D.
	31.17	246	7.24	N.D.
	31.5	251	7.69	N.D.
	36.17	235	7.26	N.D.
14	8.75	529	1.90	N.D.
	10.50	380	1.61	N.D.
	11.50	282	1.03	N.D.
	13.25	211	1.61	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
14 (Cont'd)	15.25	165	1.70	N.D.*
	17.42	121	2.11	N.D.
	32.33	41.5	1.61	N.D.
	34.00	37.6	1.90	N.D.
15	8.00	386	4.1	N.D.
	9.17	381	3.23	N.D.
	9.93	362	3.33	N.D.
	12.75	221	2.70	N.D.
	15.00	151	2.65	N.D.
	17.75	136	3.17	N.D.
	39.00	2.8	0.8	N.D.
17	4.5	613	4.40	13.4
	5.67	586	4.21	37.5
	6.75	585	2.82	39.7
	8.67	526	2.69	8.0
	14.5	302	3.01	N.D.
	19.0	211	3.09	N.D.
	23.0	149	3.39	N.D.
	27.25	115	3.71	N.D.
	43.5	5.30	1.49	N.D.
19	1.75	335	1.78	26.7
	2.75	338	1.70	13.1
	4.5	366	1.63	9.1
	5.75	277	1.38	3.1
	7.5	271	1.75	8.4
	9.5	221	1.42	3.6
	19.83	210	2.26	N.D.
20	1.83	285	1.0	3.3
	4.75	491	0.88	11.0
	6.17	441	0.78	3.4
	7.5	333	0.77	N.D.
	9.0	247	0.75	N.D.
	12.5	180	0.77	N.D.
	17.17	130	0.91	N.D.
	31.75	70.6	1.01	N.D.
21	4.0	399	3.28	N.D.
	4.67	402	3.26	N.D.
	7.0	296	2.83	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND

ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
21 (Cont'd)	8.0	296	2.45	N.D.*
	9.0	200	2.55	N.D.
	14.5	92.5	3.23	N.D.
22	9.5	397	3.59	N.D.
	10.5	358	3.01	N.D.
	11.33	375	1.92	N.D.
	12.42	289	2.35	N.D.
	13.33	245	2.31	N.D.
	14.33	162	1.93	N.D.
	15.33	125	2.50	N.D.
	16.33	118	2.54	N.D.
	18.0	101	2.51	N.D.
	36.5	7.60	1.41	N.D.
23	5.17	271	1.11	4.7
	7.5	133	1.08	3.8
	18.5	63.0	2.45	N.D.
24	8.83	506	3.48	N.D.
	10.0	455	4.22	N.D.
	12.0	436	4.01	N.D.
	14.0	330	2.90	N.D.
	16.0	287	2.72	N.D.
	25.0	283	5.30	N.D.
	26.33	263	4.47	N.D.
	30.5	265	5.64	N.D.
	49.75	143	3.62	N.D.
25	12.5	390	3.11	N.D.
	13.5	375	2.52	N.D.
	14.5	302	1.82	N.D.
	15.75	229	1.54	N.D.
	17.0	180	1.61	N.D.
	18.75	137	1.60	N.D.
	20.0	117	1.40	N.D.
	24.42	89.0	2.15	N.D.
	35.75	25.3	1.72	N.D.
	48.0	N.D.	N.D.	N.D.
	61.0	N.D.	N.D.	N.D.
26	11.83	495	3.89	N.D.
	12.83	365	2.30	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
26 (Cont'd)	13.83	312	2.04	N.D.*
	15.0	244	2.14	N.D.
	16.0	218	2.31	N.D.
	18.08	160	2.11	N.D.
	21.17	122	2.48	N.D.
	35.83	18	2.04	
41	21.5	657	6.09	N.D.
	23.5	547	3.55	N.D.
	24.75	490	3.00	N.D.
	26.5	491	2.80	N.D.
	26.75	480	2.76	N.D.
	27.5	447	2.93	N.D.
	31.5	403	2.66	N.D.
	35.5	360	2.93	N.D.
	40.33	335	3.12	N.D.
	49	295	2.74	N.D.
	63.17	178	3.25	N.D.
	67.5	173	3.65	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE (FORCED DIURESIS)

Patient No.	Hours after ingestion	Plasma concentrations (µg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
27	6	470	2.68	N.D.*
	7.33	406	2.56	N.D.
	8.5	385	2.52	N.D.
	9.33	382	2.56	N.D.
	11	342	2.25	N.D.
	13	313	2.13	N.D.
	18.5	297	2.45	N.D.
	23.25	286	2.49	N.D.
	32.5	182	2.38	N.D.
	42.66	128	2.65	N.D.
29	2.66	330	2.2	20.7
	3.66	378	2.7	25
	6	311	1.9	3.25
	7	278	1.6	N.D.
	9.25	227	1.85	N.D.
	10.92	209	1.85	N.D.
	12.5	198	2.22	N.D.
	20.25	185	2.75	N.D.
	24.25	163	4.04	N.D.
30	8.5	422	3.7	N.D.
	12	279	2.5	N.D.
	17.33	251	2.33	N.D.
31	4	540	2.65	6.78
	4.75	509	2.55	3.95
	5.92	460	2.08	6.9
	7.25	450	1.98	4.3
	8.42	384	1.88	2.6
	10	380	2.14	N.D.
	14.83	323	2.07	N.D.
	17.58	306	2.11	N.D.
	20.5	281	2.51	N.D.
	29.25	206	3.16	N.D.
	39.75	161	3.39	N.D.
32	2.67	482	2.75	6.8
	3.33	452	2.4	4.5
	4.75	362	1.95	N.D.
	6.5	303	2.04	N.D.
	8	258	2.56	N.D.
	13.17	244	2.51	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE (FORCED DIURESIS)

Patient No.	Hours after ingestion	Plasma concentrations (µg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
32 (Cont'd)	17.25	211	3.13	N.D.*
	26.5	149	3.74	N.D.
	36.92	73	2.64	N.D.
33	2	532	4.84	6.5
	3	482	4.0	2.6
	4	414	3.54	N.D.
	5	396	4.65	N.D.
	7	366	5.54	N.D.
	8.17	373	4.45	N.D.
	10.5	343	4.01	N.D.
	25	249	7.25	N.D.
	30	189	5.72	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

Alkali alone

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
37	4	475	4.2	3.1
	4.83	474	3.6	3.2
	6	459	3.2	N.D.*
	7	353	3.6	N.D.
	8	325	3.8	N.D.
	10.83	242	3.3	N.D.
	11.83	244	3.4	N.D.
	13.93	184	4.1	N.D.
	23.75	106	4.4	N.D.
38	1	394	2.7	17.4
	1.75	413	2.2	6.3
	3.25	329	1.9	N.D.
	4.25	315	2.0	N.D.
	5.25	212	2.4	N.D.
	7.75	226	2.4	N.D.
	13.25	175	2.6	N.D.
	15.42	174	2.7	N.D.
	18.58	167	2.9	N.D.
	21.75	126	3.0	N.D.
44	2.25	576	4.40	42.4
	4	514	3.86	5.68
	5	408	3.26	N.D.
	6	335	3.73	N.D.
	8.83	198	3.20	N.D.
	11.5	142	4.28	N.D.
	14	109	4.70	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

Alkali alone

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
44	18	83	5.25	N.D.*
(Cont'd)	21	36	5.40	N.D.
	31.5	5.9	2.93	N.D.
	36.66	N.D.	N.D.	N.D.
47	1.25	370	3.78	3.35
	2.75	428	3.7	6.7
	4	470	3.96	7.3
	5	419	3.25	1.63
	6	388	2.96	1.41
	8	312	3.41	N.D.
	10	325	3.58	N.D.
	12.25	270	4.14	N.D.
	16.5	204	5.83	N.D.
	26.66	102	3.91	N.D.
	38	49	4.00	N.D.
49	2	471	2.15	15.8
	3.5	355	2.04	1.23
	5	245	1.48	N.D.
	6	209	1.48	N.D.
	7	170	1.72	N.D.
	8	166	2.34	N.D.
	9	105	2.07	N.D.
	11	69	1.77	N.D.
	15	20.3	1.29	N.D.
	19	N.D.	N.D.	N.D.
	25	N.D.	N.D.	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

Alkali alone

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
51	11	346	1.25	N.D.*
	12	305	1.00	N.D.
	14	243	0.92	N.D.
	14.75	180	0.92	N.D.
	15.75	147	0.99	N.D.
	20.5	101	0.99	N.D.
	25	44	0.94	N.D.
	28	23	0.80	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

Forced Alkaline Diuresis + Frusemide

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
53	4	347	1.25	N.D.*
	5	314	1.23	N.D.
	6	260	1.33	N.D.
	7	249	1.60	N.D.
	8.25	244	2.27	N.D.
	10.67	192	1.86	N.D.
	12.92	161	1.57	N.D.
	18	79.2	2.00	N.D.
	24.5	16.3	0.94	N.D.
	32.16	N.D.	N.D.	N.D.
54	2.5	539	4.46	6.2
	4.08	529	5.40	7.1
	5.17	442	3.37	5.9
	6.83	388	4.1	5.1
	8.5	319	4.75	4.4
	10.17	279	4.90	3.2
	11.08	265	5.11	2.4
	13	252	5.56	1.33
	16.5	196	4.63	N.D.
	25	139	4.85	N.D.
	39	22.7	3.27	N.D.
	50.92	N.D.	N.D.	N.D.
55	2.25	527	5.97	7.7
	3.5	551	5.55	6.0
	4.5	450	5.12	N.D.
	6.5	349	4.71	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

Forced Alkaline Diuresis + Frusemide

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
55 (Continued)	7.5	269	3.06	N.D.*
	9	251	2.12	N.D.
	11	232	2.75	N.D.
	14	224	3.47	N.D.
	19	197	3.55	N.D.
57	24.0	449	10.4	N.D.
	25.75	492	9.6	N.D.
	27.5	354	5.7	N.D.
	29.5	262	5.0	N.D.
	30.5	240	5.9	N.D.
	31.5	205	6.7	N.D.
	35.5	176	8.8	N.D.
	39.0	99.3	7.4	N.D.
	48	10.1	4.1	N.D.
	60.67	N.D.	N.D.	N.D.
	65.25	N.D.	N.D.	N.D.
58	3	512	3.67	5.56
	4	457	3.01	1.86
	5	423	3.01	N.D.
	6	373	2.99	N.D.
	7.17	369	2.98	N.D.
	8.17	341	3.09	N.D.
	10.17	315	2.91	N.D.
	14.17	262	2.96	N.D.

* not detectable

PLASMA CONCENTRATIONS OF SALICYLIC, SALICYLURIC AND ACETYLSALICYLIC ACIDS IN PATIENTS WITH ASPIRIN OVERDOSAGE

Forced Alkaline Diuresis + Frusemide

Patient No.	Hours after ingestion	Plasma concentrations (μg/ml)		
		Salicylic acid	Salicyluric acid	Acetylsalicylic acid
58 (Cont'd)	18.17	225	2.93	N.D.*
	21	194	3.48	N.D.
	28.67	138	2.90	N.D.
	35.5	79	2.55	N.D.
	44.67	21	1.58	N.D.
59	8.25	392	3.34	3.35
	9	383	2.99	3.89
	10	359	2.66	5.82
	11	328	2.66	3.68
	12.25	308	2.93	3.01
	13.5	340	3.22	N.D.
	14.75	333	3.52	N.D.
	17	327	3.46	N.D.
	21	254	2.97	N.D.
	28	185	3.54	N.D.
	37.25	130	3.52	N.D.

* not detectable

URINARY EXCRETION DATA

CONTROL

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
1	3-6	720	6.7	326	269	70
	6-10	560	6.8	696	436	69
	10-13	450	6.4	476	562	N.D.*
	13-17	425	6.2	309	491	N.D.
	17-19	320	6.1	205	708	N.D.
	19-23	415	6.1	201	1033	N.D.
	23-31.5	970	6.1	54.2	549	N.D.
	31.5-34	195	5.6	57	928	N.D.
4	8.5-10.5	350	7.0	308	223	12.5
	10.5-12.33	350	5.4	114	511	12.1
	12.33-18.5	265	5.2	53	642	N.D.
	18.5-21.83	290	5.2	25	403	N.D.
6	2.83-4.5	790	5.4	411	332	51.5
	4.5-6	645	6.9	295	295	N.D.
	6-9	660	6.2	128	653	N.D.
	9-14	425	5.9	91	1409	N.D.
7	1-5	270	7.2	1242	669	21.2
	5-20	460	7.5	258	308	N.D.
	7.33-8.25	290	7.0	182	230	N.D.
	8.25-10	420	6.3	97	319	N.D.
	10-14	190	5.3	30.3	955	N.D.
	14-17	360	5.4	13.3	592	N.D.
	17-20	375	5.5	7.0	258	N.D.
8	N.A.**	N.A.	N.A.	N.A.	N.A.	N.A.
9	8-11.25	629	6.4	233	214	N.D.
	11.25-22.5	365	5.6	257	944	N.D.
	22.5-26.75	550	5.7	91.3	561	N.D.

* not detectable ** not available

URINARY EXCRETION DATA

CONTROL

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
13	2-2.83	240	6.5	296	382	184
	2.83-3.16	340	6.5	177	176	40
	3.16-4.66	250	7.0	393	258	18.5
	4.66-5.66	300	7.1	738	325	5.5
	5.66-6.08	300	7.1	341	151	N.D.*
	6.08-7.42	310	7.1	296	114	N.D.
	7.42-8.16	150	7.2	839	348	N.D.
	8.16-9.33	80	7.2	1966	1172	N.D.
	9.33-13.42	50	6.6	1284	3200	N.D.
	13.42-15.92	55	6.5	957	2954	N.D.
	15.92-16.66	40	6.1	409	2910	N.D.
16	4-7.33	410	5.9	32.15	389	N.D.
	7.33-14.66	40	6.0	42.18	2355	N.D.
	14.66-31.25	565	6.0	186	1982	N.D.
34	20-22	210	6.9	208	386	N.D.
	22-1.75	60	7.0	1688	1957	N.D.
	1.75-5.5	150	6.7	644	1178	N.D.
	5.5-9	260	6.3	111	588	N.D.
	9-10.75	240	7.2	237	605	N.D.
	10.75-12	50	6.4	96	1921	N.D.
	12-14.25	60	6.6	288	1945	N.D.
35	1-7	660	6.5	100	733	N.D.
	7-11.5	270	6.4	118	1746	N.D.
	11.5-15.75	155	6.3	104	2119	N.D.
	15.75-17.92	130	5.8	25	1863	N.D.
	17.92-20	130	6.2	48	1411	N.D.

* not detectable

URINARY EXCRETION DATA

CONTROL

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
36	8-18	285	7.1	1211	1610	N.D.*
	18-30	250	6.4	203	2443	N.D.
	30-35.58	130	5.4	50	2283	N.D.
43	14.66-18.5	300	5.8	62.5	1098	N.D.
	18.5-22	265	6.1	23.2	1072	N.D.
	22-24.5	140	6.1	33.0	1867	N.D.
	24.5-27	160	6.1	48.3	1437	N.D.
	27-30	360	6.4	37.8	1314	N.D.
	30-34.5	135	6.0	24.0	833	N.D.
	34.5-36.5	290	5.9	48.1	2729	N.D.
	36.5-38.5	430	6.1	28.8	733	N.D.
	38.5-41.66	220	6.7	49.0	406	N.D.
	41.66-43.83	460	6.9	255	1179	N.D.
	43.83-47	370	7.1	143	840	N.D.
	47-49.5	740	7.0	235	692	N.D.
	49.5-52.5	400	6.4	84.8	294	N.D.
	52.5-58.5	920	6.0	34.1	727	N.D.
	58.5-60	360	6.7	14.7	442	N.D.
45	0-14.5	590	6.6	643	622	N.D.
	14.5-23.5	97	5.7	262	1032	N.D.
	23.5-30.5	660	5.8	196	994	N.D.
	30.5-36.5	910	5.8	96.3	640	N.D.
	36.5-38.5	555	5.0	80.5	767	N.D.
48	4.5-9	420	6.2	331	462	N.D.
	9-15.66	250	6.0	359	2199	N.D.
	15.66-20.25	130	5.7	96.0	4662	N.D.

* not detectable

URINARY EXCRETION DATA

CONTROL

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
48	15.66-20.25	130	5.7	96.0	4662	N.D.*
(contd)	20.25-27.5	315	5.7	64.0	1837	N.D.
	27.5-34.75	490	5.8	51.4	1104	N.D.
	34.75-36	280	6.5	60.0	374	N.D.
50	12-15	150	6.6	203	865	N.D.
	15-18.25	75	6.6	237	5229	N.D.
	18.25-24.5	70	6.4	378	3460	N.D.
	24.5-35	145	6.3	271	3030	N.D.
	35-36	260	6.8	16.4	239	N.D.
	36-38	140	6.6	24.4	321	N.D.
	38-38.5	215	6.3	78.0	848	N.D.
	38.5-41.25	60	6.3	82.3	858	N.D.
52	5.5-10.5	480	6.1	422	1098	88.6
	10.5-19.75	260	5.7	107	1850	N.D.
	19.75-27.25	195	5.8	91.1	2598	N.D.
	27.25-39	290	5.8	74.1	2964	N.D.
	39-41.5	80	5.8	31.2	2815	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
2	7.75-8.5	475	7.6	1270	246	14.8
	8.5-9.5	1120	7.7	783	78	N.D.*
	9.5-10.25	1220	7.7	438	40	N.D.
	10.25-15.5	1680	7.8	756	123	N.D.
	15.5-20.75	420	7.6	578	710	N.D.
	20.75-31	540	7.5	82	200	N.D.
	31-33	520	6.7	N.D.	53	N.D.
	33-39	1300	6.7	N.D.	220	N.D.
3	4.5-7.25	1050	7.5	551	103	N.D.
	7.25-7.75	450	7.5	434	39.8	N.D.
	7.75-8.25	490	7.7	406	37.3	N.D.
	8.25-9.0	530	7.9	446	69.8	N.D.
	9 - 10	355	7.9	614	128	N.D.
	10 -21	370	7.9	1571	1304	N.D.
	21-25.25	70	8.5	2543	1876	N.D.
5	4.25-6.5	730	7.4	672	219	3.8
	6.5-7.17	710	7.4	505	116	N.D.
	7.17-7.25	205	7.3	495	120	N.D.
	7.25-8.08	1150	7.7	553	102	N.D.
	8.08-9.5	850	7.7	741	180	N.D.
	9.5-12.5	590	7.3	911	310	N.D.
	12.5-15.0	420	7.1	1162	820	N.D.
	15.0-19.75	530	7.8	645	515	N.D.
	19.75-23.25	250	7.5	117	1758	N.D.
	23.25-36.5	585	6.8	229	1938	N.D.
10	15.5-19.0	315	7.4	4774	1445	111
	19-20.83	400	7.8	3935	548	12.5
	20.83-24.25	350	7.9	4710	1097	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
10	24.25-29	790	6.9	516	536	N.D.*
(Cont'd)	29-31	610	6.9	339	408	N.D.
	31-35	320	6.9	91.3	179	N.D.
	35-36.5	710	6.7	97.8	180	N.D.
14	5.75-10	410	7.5	2724	416	91.3
	10-10.5	555	7.5	838	35.7	N.D.
	10.5-11	550	6.8	820	45.2	N.D.
	11-11.75	650	7.7	759	42.8	N.D.
	11.75-12.17	675	7.6	503	27.8	N.D.
	12.17-12.58	675	7.8	424	21.5	N.D.
15	7.0-10.5	150	6.9	3355	2339	N.D.
	10.5-11.5	810	7.5	1137	143	N.D.
	11.5-12.83	890	6.9	852	134	N.D.
17	3.0-5.0	690	7.1	527	193	348
	5.0-6.17	630	7.4	875	278	272
	6.17-6.5	710	7.5	895	124	131
	6.5-7.17	630	7.6	803	87.8	87.7
	7.17-8.5	570	8.2	1459	124	71.8
	8.5-16.5	710	8.0	2531	439	17.7
	16.5-22.5	290	7.2	3143	1877	N.D.
	22.5-23.75	120	6.8	888	2221	N.D.
19	3.25-3.93	500	7.3	528	59.7	53.3
	3.93-4.5	710	7.4	624	60.5	52.7
	4.5-4.75	570	7.5	494	26.5	8.4
	4.75-5.0	560	7.5	652	35.5	17.2
	5.0-5.5	680	7.5	586	34.5	6.0
	5.5-5.75	560	7.6	466	28.4	N.D.
	5.75-6.0	710	7.6	391	23.0	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
19	6.0-6.75	500	7.6	533	49.0	N.D.*
(Cont'd)	6.75-7.75	440	7.7	1260	187	N.D.
	7.75-8.83	350	7.6	997	143	N.D.
	8.83-17.0	415	7.9	2649	997	N.D.
	17.0-19.5	210	7.9	1307	593	N.D.
20	4.0-6.0	320	7.4	1186	73.1	31.1
	6.0-6.25	345	7.5	792	22.0	6.8
	6.25-6.58	360	7.7	603	20.0	5.8
	6.58-7.0	290	7.7	803	30.5	N.D.
	7.0-7.42	210	7.9	767	23.1	N.D.
	7.42-7.58	240	7.8	601	19.0	N.D.
	7.58-7.83	660	7.8	495	13.7	N.D.
	7.83-8.17	405	7.9	504	14.3	N.D.
	8.17-8.5	245	7.9	708	23.4	N.D.
	8.5-9.08	255	8.1	1081	40.0	N.D.
	9.08-10.25	320	8.2	1636	89.0	N.D.
	10.25-16.25	325	7.5	1861	378	N.D.
	16.25-26.5	220	8.1	768	598	N.D.
21	5.0-6.5	790	6.4	1238	585	91.0
	6.5-7.5	690	8.0	1615	201	29.1
	7.5-8.5	620	8.0	947	92.1	N.D.
	8.5-9.0	740	8.0	800	78.0	N.D.
	9.0-10.0	245	8.1	617	129	N.D.
22	8.0-12.25	660	7.3	1316	421	17.4
	12.25-12.5	630	7.9	430	52.0	N.D.
	12.5-12.83	540	7.8	340	31.3	N.D.
	12.83-13.25	565	7.8	378	38.0	N.D.
	13.25-13.83	540	7.8	453	57.4	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
22 (Cont'd)	13.83-14.08	500	7.9	342	50.1	N.D.*
	14.08-14.58	510	7.9	402	69.6	N.D.
	14.58-15.17	410	8.0	483	104	N.D.
	15.17-20.5	440	7.5	743	593	N.D.
	20.5-32.17	445	7.0	214	1132	N.D.
	32.17-33.17	250	7.1	25.5	115	N.D.
23	0-5.33	600	7.6	1652	139	288
	5.33-5.75	570	7.9	929	51.0	119
	5.75-6.17	630	8.2	794	64.2	11.6
	6.17-6.75	740	8.4	699	65.0	N.D.
	6.75-7.5	595	8.2	798	68.0	N.D.
	7.5-9.5	670	8.3	945	140	N.D.
24	1.25-9.25	330	6.1	1.858	2752	197
	9.25-14.0	1020	7.2	1693	478	N.D.
	14.0-15.75	1250	7.5	675	140	N.D.
	15.75-19.25	1010	6.8	547	393	N.D.
	19.25-27.5	570	5.2	248	1490	N.D.
	27.5-30.58	560	6.0	233	681	N.D.
	30.58-47.0	475	5.6	587	6977	N.D.
25	11.0-14.25	475	7.1	2380	1200	N.D.
	14.25-15.33	690	7.5	797	99.7	N.D.
	15.33-15.92	475	7.8	858	103	N.D.
	15.92-17.0	590	8.1	1020	108	N.D.
	17.0-18.58	505	8.1	1431	193	N.D.
	18.58-22.83	268	7.9	1703	918	N.D.
	22.83-32.0	510	6.5	202	1188	N.D.
	32.0-33.83	80	8.1	601	746	N.D.
	33.83-35.83	615	7.7	158	218	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
25	35.83-38.0	190	8.1	181	534	N.D.*
(Cont'd)	38.0-41.0	370	7.4	60.0	278	N.D.
	41.0-42.83	355	7.4	13.2	68.0	N.D.
	42.83-44.0	230	7.5	12.0	82.8	N.D.
	44.0-46.5	230	7.4	10.0	88.0	N.D.
	46.5-50.5	200	7.4	N.D.	42.7	
	50.5-58.0	270	7.4	N.D.	13.8	N.D.
	58.0-61.17	810	7.5	N.D.	4.8	N.D.
26	10.5-12.25	855	7.2	1020	490	N.D.
	12.25-12.75	705	7.6	570	119	N.D.
	12.75-13.25	800	7.8	567	92.5	N.D.
	13.25-14.0	840	7.9	469	75.2	N.D.
	14.0-14.5	860	7.9	463	96.0	N.D.
	14.5-16.5	705	7.9	570	305	N.D.
	16.5-18.5	285	7.2	559	340	N.D.
	18.5-20.5	210	7.0	237	838	N.D.
	20.5-22.0	320	6.9	89.0	347	N.D.
	22.0-23.25	470	7.4	60.0	227	N.D.
	23.25-34.0	320	6.6	12.8	528	N.D.
41	18.5-26.17	580	6.2	1267	3363	N.D.
	26.17-26.0	480	7.2	1253	147	N.D.
	26.0-27.5	530	7.0	860	171	N.D.
	27.5-30.5	540	7.8	776	291	N.D.
	30.5-31.0	280	6.7	725	424	N.D.
	31.0-33.0	330	6.3	415	529	N.D.
	33.0-35.5	350	6.2	347	509	N.D.
	35.5-41.5	400	5.7	221	681	N.D.
	41.5-47.5	350	5.8	278	1123	N.D.
	47.5-51.0	340	6.5	969	1168	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
41	51.0-52.5	255	6.8	1140	792	N.D.*
(Cont'd)	52.5-56.25	290	6.2	489	873	N.D.
	56.25-58.5	245	6.1	434	1180	N.D.
	58.5-67.5	130	5.4	114	1799	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
27	6-8.5	240	6.1	571	923	17.5
	8.5-9.83	230	6.0	386	749	13
	9.83-10.75	270	6.0	359	620	11
	10.75-11.67	350	6.0	292	391	N.D.*
	11.67-12.67	210	5.8	260	438	N.D.
	12.67-13.75	215	5.8	192	403	N.D.
	13.75-14.67	380	5.9	128	320	N.D.
	14.67-15.5	390	5.9	81	252	N.D.
	15.5-18	720	5.9	49	390	N.D.
	18-19	310	6.0	57.3	402	N.D.
	19-19.75	530	6.1	64	199	N.D.
	19.75-21	570	6.3	101	322	N.D.
	21-22	285	6.3	115	355	N.D.
	22-23	355	6.6	162	289	N.D.
	23-24	495	6.9	340	362	N.D.
	24-25.33	450	6.8	244	214	N.D.
	25.33-26.75	175	6.6	187	382	N.D.
	26.75-27.5	150	5.8	100.	819	N.D.
	27.5-30.42	560	5.6	30	451	N.D.
	30.42-32.67	345	5.4	18.8	576	N.D.
	32.67-40	280	5.4	43.7	1662	N.D.
	40-41	310	5.6	45.7	493	N.D.
	41-42.67	140	5.4	13.7	982	N.D.
29	0-3.5	395	7.3	322	133	246
	3.5-6	920	7.3	316	74.5	N.D.
	6-8.25	730	7.6	326	56.5	N.D.
	8.25-13	400	6.8	330	511	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
29 (cont'd)	13-16	375	5.8	74.2	513	N.D.*
	16-17.5	415	5.7	20	238	N.D.
	17.5-19	500	5.7	13.5	150	N.D.
	19-24.5	450	5.2	17.9	374	N.D.
30	5-9	470	6.3	110	367	N.D.
	9-9.75	330	6.7	465	785	N.D.
	9.75-10.75	660	6.9	160	116	N.D.
	10.75-11.5	670	7.0	154	107	N.D.
	11.5-12.17	550	7.0	133	73.6	N.D.
	12.17-12.75	310	7.2	129	87.5	N.D.
	12.75-17.33	450	6.2	233	656	N.D.
31	1-7.33	890	7.8	2517	531	62.8
	7.33-9	680	7.7	1494	224	29.6
	9-14	850	6.7	426	530	N.D.
	14-16	840	6.8	133	184	N.D.
	16-17	930	7.0	139	144	N.D.
	17-20	750	6.6	146	141	N.D.
	20-32.5	620	5.9	71.7	1459	N.D.
	32.5-39.75	250	5.7	51.8	2953	N.D.
32	0-4	470	7.1	781	250	N.D.
	4-5	540	7.3	510	131	N.D.
	5-5.75	700	7.1	583	184	N.D.
	5.75-6.5	710	6.8	467	120	N.D.
	6.5-7	740	6.9	365	91	N.D.
	7-8.17	648	7.2	292	94.6	N.D.
	8.17-12.75	350	5.6	115	629	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED DIURESIS

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
32	12.75-14.33	415	6.3	159	249	N.D.*
(Cont'd	14.33-16.33	745	5.8	152	325	N.D.
	16.33-20.25	540	5.8	133	387	N.D.
	20.25-23.75	470	4.7	130	652	N.D.
	23.75-26	320	5.5	107	956	N.D.
	26-31	140	5.3	63.6	1678	N.D.
	31-33.58	95	5.4	53.3	2135	N.D.
	33.58-36.75	80	5.6	73.1	2019	N.D.
33	0-3.5	370	6.9	669	517	160
	3.5-4.5	230	7.8	551	201	58.4
	4.5-6.5	320	7.2	463	199	68.8
	6.5-8	1360	6.9	350	252	21.8
	8-10	175	5.5	113	873	N.D.
	10-12	260	5.4	58	1546	N.D.
	12-14.83	1030	5.9	25.8	204	N.D.
	14.83-19.5	1600	6.0	10	236	N.D.
	19.5-21.33	545	5.9	9	315	N.D.
	21.33-24.5	225	5.3	12	964	N.D.
	24.5-26.5	50	5.5	62.5	3313	N.D.
	26.5-30	80	5.5	80	4059	N.D.

* not detectable

URINARY EXCRETION DATA

ALKALI ALONE

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
37	2.5-5	550	7.0	267	326	130
	5-5.75	660	7.8	868	222	N.D.*
	5.75-6.33	470	7.6	915	107	N.D.
	6.33-8	500	7.8	1597	265	N.D.
	8-9	390	8.0	2081	348	N.D.
	9-11	665	7.8	891	222	N.D.
	11-13.83	380	7.5	1090	382	N.D.
	13.83-20	320	7.9	1023	1594	N.D.
	20-23.75	150	7.0	279	2033	N.D.
38	1.25-2.75	490	7.2	547	141	N.D.
	12.75-3.25	770	7.3	444	89.4	N.D.
	3.25-4.75	580	7.8	1232	194	N.D.
	4.75-11.25	430	7.6	2103	1231	N.D.
	11.25-17.67	130	6.9	747	1548	N.D.
	17.67-21.75	230	6.5	133	876	N.D.
44	1 - 2.25	235	6.7	269	103	233
	2.25-3.83	520	7.5	1030	135	N.D.
	3.83-4.75	710	8.0	1738	275	N.D.
	4.75-6	615	8.4	2334	205	N.D.
	6 - 7.75	345	8.6	3401	476	N.D.
	7.75-10.93	176	8.4	4014	1483	N.D.
	10.93-13	66	8.3	2673	2640	N.D.
	13 -19.75	320	6.6	182	1624	N.D.
	19.75-22	117	5.7	217	1229	N.D.
	22-22.5	140	5.9	237	1328	N.D.
	22.5-29	180	6.1	22	2162	N.D.
47	?-4	610	7.3	825	593	175
	4-7.5	460	8.4	4551	719	56
	7.5-11.5	450	7.8	2432	740	N.D.

* not detectable

URINARY EXCRETION DATA

ALKALI ALONE

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
47	11.5-13.5	500	7.5	733	359	N.D.*
(Cont'd)	13.5-15.5	340	7.7	910	543	N.D.
	15.5-17.66	360	8.0	1520	829	N.D.
	17.66-21	400	7.6	435	597	N.D.
	21-23.5	285	7.4	306	853	N.D.
	23.5-27.5	240	7.4	288	1636	N.D.
	27.5-30	200	7.4	144	1946	N.D.
	30-32	260	7.3	78.0	607	N.D.
	32-35.5	210	7.4	90.0	663	N.D.
	35.5-38	640	7.8	98.0	621	N.D.
49	0.75-3.75	940	8.0	1213	208	232
	3.75-5.5	890	8.5	1776	299	25.5
	5.5-9.83	650	8.9	2782	682	N.D.
	9.83-17.5	570	8.8	875	939	N.D.
	17.5-25	410	8.6	10.1	197	N.D.
	25-26.5	300	8.1	N.D.	14.7	N.D.
	26.5-35.5	370	7.9	N.D.	31.6	N.D.
	35.5-39	290	7.7	N.D.	14.7	N.D.
51	8-12.33	735	7.5	822	366	N.D.
	12.33-18	640	8.7	2820	763	N.D.
	18 - 25	270	8.5	623	864	N.D.

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS PLUS FRUSEMIDE

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
53	? - 4.33	705	8.0	956	328	30.1
	4.33-5.17	1110	8.1	444	69.3	N.D.*
	5.17-5.5	1430	8.2	383	64.5	N.D.
	5.5-5.83	650	8.3	381	44.8	N.D.
	5.83-6.17	420	8.3	421	53.5	N.D.
	6.17-8.75	520	8.4	400	281	N.D.
	8.75-14.5	420	7.9	1435	1236	N.D.
	14.5 - 21	390	7.6	602	1102	N.D.
	21 - 32	240	6.2	81	1290	N.D.
54	? - 3	460	7.0	1450	145	370
	3 - 5.33	900	7.4	1457	295	360
	5.33-5.75	1020	7.4	884	118	39.4
	5.75-6.5	920	7.5	600	70.8	N.D.
	6.5-7.25	730	7.7	705	63	N.D.
	7.25-7.67	666	7.7	634	54.8	N.D.
	7.67-8.67	1090	7.8	680	75	N.D.
	8.67-13.83	690	7.3	430	668	N.D.
	13.83-23.25	280	5.8	337	2395	N.D.
	23.25-34.5	320	6.3	180	1150	N.D.
	34.5-38.83	450	6.5	92	795	N.D.
	38.83-45.75	530	6.3	12.0	235	N.D.
	45.75-50.5	220	7.2	4.0	53.3	N.D.
55	? - 2.75	760	5.8	166	160	359
	2.75-3.93	820	7.1	479	197	104
	3.93-4.43	800	7.5	732	73.5	13.1
	4.43-4.66	650	7.6	570	62.0	6.8

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS PLUS FRUSEMIDE

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
55	4.66-5.0	560	7.4	300	45.1	N.D.*
(Cont'd)	5.0 - 5.5	630	7.5	378	63.0	N.D.
	5.5 - 6.0	560	7.6	459	72.1	N.D.
	6.0 - 6.5	600	7.6	376	77.8	N.D.
	6.5 - 7.5	630	7.6	675	139	N.D.
	7.5 - 10.0	520	7.7	436	250	N.D.
	10.0-14.0	320	7.4	902	1259	N.D.
	14 - 17.33	500	7.3	732	642	N.D.
	17.33 - 19	130	7.0	8.33	1193	N.D.
57	0 - 0.5	95	5.5	62.1	5092	N.D.
	0.5 - 3.0	915	6.3	175	453	N.D.
	3.0 - 3.5	830	7.0	189	66.6	N.D.
	3.5 - 4.07	610	7.3	229	75.8	N.D.
	4.07 - 4.5	650	7.4	254	91.8	N.D.
	4.5 - 4.83	1060	7.4	241	74.1	N.D.
	4.83 -5.33	940	7.6	247	78.5	N.D.
	5.33-6.5	930	7.4	229	167	N.D.
	6.5-11.25	530	6.3	225	1317	N.D.
	11.25-14.0	100	7.5	924	3426	N.D.
	14.0-16.75	150	7.9	966	2488	N.D.
	16.75-25.17	370	7.3	316	2181	N.D.
	25.17 - 34	172	7.5	35.2	1620	N.D.
	34 - 37.17	45	8.4	N.D.	222	N.D.
	37.17 - 40	110	8.7	N.D.	47.2	N.D.
58	? - 4.17	270	6.4	1074	1319	139
	4.17-4.67	475	6.7	582	377	44.4
	4.67-5.17	970	7.0	540	149	13.4

* not detectable

URINARY EXCRETION DATA

FORCED ALKALINE DIURESIS PLUS FRUSEMIDE

Patient No.	Hours after ingestion	Volume (ml)	pH	Urine concentrations (µg/ml)		
				Salicylic acid	Salicyluric acid	Acetylsalicylic acid
58 (Cont'd)	5.17-5.67	940	7.4	570	73	N.D.*
	5.67-5.93	650	7.4	635	65.4	N.D.
	5.93-6.43	1190	7.5	698	51	N.D.
	6.43-7.67	1130	7.4	600	98.2	N.D.
	7.67-9.67	510	7.4	616	283	N.D.
	9.67-14.17	390	7.1	502	617	N.D.
	14.17-18.17	240	6.7	325	984	N.D.
	18.17-23.67	230	6.2	115	1356	N.D.
	23.67-27.93	160	6.2	53.2	1652	N.D.
	27.93-30.67	165	6.8	181	1587	N.D.
	30.67-33.67	500	7.1	71.2	717	N.D.
	33.67-38.67	250	7.1	122	1005	N.D.
	38.67-42.17	175	6.5	63.2	2644	N.D.
	42.17-45.67	285	6.7	15.4	627	N.D.
59	? - 7.83	360	7.9	2442	678	213
	7.83-10.33	1020	7.8	472	33.5	15.2
	10.33-11.17	2050	8.2	306	23.6	8.2
	11.17-12.5	850	8.1	539	51.3	N.D.
	12.5-15.0	1320	8.5	595	84.5	N.D.
	15.0 -18.5	410	8.6	2289	791	N.D.
	18.5-24.0	685	7.5	761	576	N.D.
	24 - 27.93	560	7.4	339	417	N.D.
	27.93-34.83	630	6.7	100	608	N.D.
	34.83-37.0	550	6.9	42.3	261	N.D.
	37.0-39.25	290	6.6	47.8	282	N.D.

* not detectable

URINARY SODIUM, POTASSIUM AND OSMOLALITY

Treatment	Patient No.	Sodium (mmol/24h)	Potassium (mmol/24h)	Osmolality (mmol/kg)
Control	36	15.1	68	427
	43	118	51	348
	45	37.6	91	434
	48	41.1	44	462
	50	40.2	18	405
	52	47.5	35	724
Forced alkaline diuresis	20	284	145	241
	22	410	125	292
	24	112	153	441
	25	201	57	324
	26	420	324	193
	41	120	84	486
Forced diuresis	27	255	203	341
	29	152	136	179
	30	283	343	279
	31	154	158	319
	32	252	158	346
	33	177	157	323
Alkali alone	37	273	176	455
	38	333	143	457
	44	258	122	441
	47	196	81	426
	49	296	71	308
	51	277	150	434
Forced alkaline diuresis + frusemide	53	489	212	379
	54	341	115	337
	55	633	296	306
	57	424	77	421
	58	362	197	422
	59	596	177	236

PLASMA ELECTROLYTES, OSMOLALITY, TOTAL CO2, ALBUMIN AND URATE CONCENTRATIONS ON ADMISSION

Treatment	Patient No.	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Phosphate (mmol/l)	Magnesium (mmol/l)	Osmolality (mmol/kg)	Total CO_2 (mmol/l)	Albumin (g/l)	Urate (mmol/l)
Control	36	139	7.5	2.30	1.14	0.77	332	22	44	0.26
	43	146	4.6	2.53	1.05	0.88	292	25	54	0.21
	45	146	3.8	2.59	1.46	0.86	294	23	49	0.22
	48	143	6.5	2.53	1.36	0.89	349	22	44	0.26
	50	142	3.5	2.41	1.14	0.85	283	23	44	0.11
	52	143	5.1	3.15	1.04	0.94	332	23	50	0.40
Forced alkaline diuresis	20	142	3.7	2.49	1.05	0.98	282	26	51	0.31
	22	139	3.6	2.49	1.12	0.74	295	23	45	0.19
	24	141	6.4	2.50	1.49	0.82	289	18	52	0.19
	25	143	3.3	2.42	1.24	0.87	289	19	42	0.24
	26	142	4.4	2.11	1.17	0.77	297	19	46	0.17
	41	135	4.3	2.16	1.61	1.02	298	14	46	0.22
Forced diuresis	27	141	4.4	2.43	1.54	0.78	287	22	52	0.38
	29	142	4.4	2.49	1.05	0.98	283	24	49	0.12
	30	142	5.3	2.29	1.46	1.02	341	23	50	0.29
	31	145	4.5	2.52	1.12	0.92	297	27	52	0.29
	32	139	4.3	2.11	1.11	0.67	274	21	48	0.15
	33	145	4.3	2.75	1.17	0.88	283	25	44	0.29

Alkali alone /

PLASMA ELECTROLYTES, OSMOLALITY, TOTAL CO2, ALBUMIN AND URATE CONCENTRATIONS ON ADMISSION (CONTINUED)

Treatment	Patient No.	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Phosphate (mmol/l)	Magnesium (mmol/l)	Osmolality (mmol/kg)	Total CO_2 (mmol/l)	Albumin (g/l)	Urate (mmol/l)
Alkali	37	142	4.4	2.43	1.19	0.88	294	24	45	0.26
alone	38	138	5.06	2.77	1.18	0.87	295	23	50	0.35
	44	142	5.8	2.94	1.03	1.06	310	16	45	0.20
	47	144	6.1	2.44	1.41	0.98	315	20	49	0.29
	49	148	4.7	2.25	1.13	0.78	341	27	38	0.26
	51	137	4.3	2.41	1.29	0.81	278	23	44	0.18
Forced	53	143	4.0	2.54	0.85	0.95	346	24	50	0.33
alkaline	54	139	4.5	2.45	1.00	0.92	293	21	40	0.40
diuresis	55	137	3.7	2.37	1.44	1.06	320	15	51	0.31
+	57	136	3.3	1.94	0.72	0.87	287	15	41	0.11
frusemide	58	137	5.3	2.30	1.41	0.81	323	17	39	0.10
	59	142	4.1	2.30	1.07	0.76	295	24	41	0.12

PLASMA ELECTROLYTES, OSMOLALITY, TOTAL CO_2, ALBUMIN AND URATE CONCENTRATIONS AT THE END OF INFUSION (4 HOURS AFTER ADMISSION)

Treatment	Patient No.	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Phosphate (mmol/l)	Magnesium (mmol/l)	Osmolality (mmol/kg)	Total CO_2 (mmol/l)	Albumin (g/l)	Urate (mmol/l)
Control	36	139	4.8	2.11	1.12	0.68	293	23	39	0.15
	43	143	4.2	2.28	1.25	0.87	284	24	46	0.20
	45	139	6.1	2.18	1.61	0.84	286	20	40	0.13
	48	145	5.2	2.19	1.16	0.79	331	21	42	0.20
	50	142	3.8	2.38	1.46	0.84	283	21	42	0.13
	52	144	3.7	2.61	1.12	0.96	295	20	43	0.31
Forced alkaline diuresis	20	143	3.4	1.94	0.50	0.84	284	27	41	0.11
	22	141	3.3	2.21	1.11	0.64	287	28	39	0.12
	24	144	3.3	2.34	0.98	0.74	284	24	42	0.12
	25	143	3.3	1.90	0.58	0.73	290	31	35	0.09
	26	135	3.6	1.95	0.33	0.64	287	27	39	0.15
	41	129	2.9	1.74	0.42	0.87	284	19	32	0.14
Forced diuresis	27	132	6.0	2.06	0.86	0.67	277	18	45	0.25
	29	142	4.2	2.10	0.82	0.87	273	20	41	0.07
	30	138	5.1	1.92	0.69	0.89	305	20	39	0.19
	31	139	3.7	2.15	1.04	0.80	269	23	43	0.11
	32	137	5.6	2.16	0.96	0.66	271	19	38	0.14
	33	124	5.1	2.05	0.52	0.63	258	16	40	0.18

Alkali alone/

PLASMA ELECTROLYTES, OSMOLALITY, TOTAL CO2, ALBUMIN AND URATE CONCENTRATIONS AT THE END OF INFUSION (4 HOURS AFTER ADMISSION) (CONTINUED)

Treatment	Patient No.	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Phosphate (mmol/l)	Magnesium (mmol/l)	Osmolality (mmol/kg)	Total CO_2 (mmol/l)	Albumin (g/l)	Urate (mmol/l)
Alkali	37	144	4.0	2.16	0.96	0.81	292	29	42	0.21
alone	38	142	4.7	2.43	1.38	0.80	292	30	46	0.25
	44	146	4.6	2.47	0.96	0.82	291	26	43	0.13
	47	145	5.7	2.15	1.42	0.86	298	26	44	0.23
	49	149	4.1	1.96	0.96	0.66	321	33	33	0.18
	51	137	5.0	2.13	1.09	0.77	283	26	39	0.13
Forced	53	136	3.6	2.13	0.81	0.49	288	31	48	0.19
alkaline	54	134	4.3	2.07	0.76	0.84	290	21	37	0.34
diuresis	55	140	3.2	2.12	1.15	0.78	284	24	41	0.18
+	57	135	3.8	1.88	0.34	0.67	287	25	43	0.09
frusemide	58	127	4.4	1.80	0.55	0.57	299	27	33	0.06
	59	133	3.4	1.93	0.27	0.60	285	26	43	0.10

PLASMA ELECTROLYTES, OSMOLALITY, TOTAL CO2, ALBUMIN AND URATE CONCENTRATIONS AT 16 HOURS AFTER ADMISSION

Treatment	Patient No.	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Phosphate (mmol/l)	Magnesium (mmol/l)	Osmolality (mmol/kg)	Total CO_2 (mmol/l)	Albumin (g/l)	Urate (mmol/l)
Control	36	123	3.8	1.93	0.68	0.69	286	22	40	0.11
	43	144	4.0	2.39	0.52	0.74	286	28	45	0.17
	45	140	3.5	2.19	0.70	0.87	287	22	40	0.10
	48	140	3.9	2.07	1.06	0.82	284	25	38	0.11
	50	141	3.7	2.38	1.14	0.83	285	23	40	0.13
	52	141	3.9	2.27	0.70	0.94	295	23	41	0.25
Forced alkaline diuresis	20	143	3.7	2.34	0.81	0.98	282	26	44	0.14
	22	140	4.3	2.43	0.71	0.74	294	27	44	0.17
	24	149	4.4	2.48	0.62	0.65	303	27	42	0.12
	25	140	3.3	2.29	0.80	0.81	287	25	39	0.12
	26	141	3.5	2.45	0.67	0.79	281	29	40	0.17
	41	138	2.8	1.95	0.57	0.92	285	24	35	0.09
Forced diuresis	27	135	3.8	2.15	0.71	0.74	279	21	44	0.12
	29	143	3.8	2.33	0.90	0.88	272	22	41	0.06
	30	140	4.0	2.08	0.60	0.83	280	21	43	0.15
	31	131	4.4	1.98	0.65	0.67	258	21	44	0.17
	32	146	4.5	2.83	0.61	0.84	299	20	47	0.21
	33	146	3.5	2.43	0.61	0.63	281	22	43	0.15

Alkali alone/

PLASMA ELECTROLYTES, OSMOLALITY, TOTAL CO2, ALBUMIN AND URATE CONCENTRATIONS AT 16 HOURS AFTER ADMISSION (CONTINUED)

Treatment	Patient No.	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Phosphate (mmol/l)	Magnesium (mmol/l)	Osmolality (mmol/kg)	Total CO_2 (mmol/l)	Albumin (g/l)	Urate (mmol/l)
Alkali alone	37	141	3.9	2.29	0.70	0.88	282	26	44	0.12
	38	142	3.4	2.38	0.93	0.86	287	26	45	0.21
	44	139	3.0	2.17	1.21	0.76	276	24	36	0.11
	47	143	4.2	2.24	0.75	0.95	281	27	41	0.16
	49	142	3.8	2.33	0.61	0.76	185	24	37	0.24
	51	138	3.9	2.30	0.81	0.80	282	24	42	0.15
Forced alkaline diuresis + frusemide	53	137	3.3	2.34	0.69	0.80	281	30	43	0.23
	54	140	4.4	2.03	0.61	0.93	264	25	37	0.31
	55	141	3.2	2.40	1.02	0.43	287	26	44	0.14
	57	141	4.2	2.38	0.69	0.91	288	24	44	0.14
	58	138	2.7	2.11	0.28	0.74	279	28	30	0.08
	59	140	3.0	2.19	0.82	0.70	286	27	37	0.08

APPENDIX II

PUBLISHED PAPERS RELATING TO THIS THESIS

niques are leading to a rapid increase in the standard of analyses which the laboratory can offer to the ward.

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HPLC in Clinical Pharmacological Studies of Analgesic Drugs

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Most of the methods used for the assay of analgesics such as aspirin and paracetamol in biological fluids are non-specific and there are major difficulties with the determination of their metabolites. Gas - liquid chromatographic methods overcome the problems of specificity, but with therapeutic concentrations of these drugs derivatisation is necessary because of their polar nature. The introduction of HPLC has been a great advance and it is now possible to separate and measure many polar drugs and metabolites by using simple procedures.

We have developed simple, rapid and specific HPLC assays for aspirin, salicylic acid, paracetamol, antipyrine, diflunisal, phenylbutazone and mefenamic acid in plasma and urine. With aspirin and paracetamol, metabolites can be measured simultaneously. No solvent extraction is required and the whole procedure can usually be completed within a few minutes.

The general method for the determination of these drugs in plasma consists of the addition of a suitable internal standard, precipitation of plasma proteins with perchloric acid or acetone, centrifugation and direct injection of 5–10 μl aliquots of the clear supernatant into the HPLC column. The methods for urine are even simpler. The aqueous internal standard is added and an aliquot of the mixture injected into the column.

The columns were internally polished stainless steel, 100–150 × 4.5 mm i.d., packed with 5 μm C_{18}-bonded spherical silica (Hypersil-ODS or Spherisorb-ODS) and fitted with septum injectors. An Orlita pump (Model AE 10-4) was used with a Cecil Model 212, Pye Model LC3 or Waters Model 440 ultraviolet detector, a strip-chart recorder and an integrator. The solvent was aqueous propan-2-ol containing combinations of formic or acetic acid, potassium nitrate, potassium dihydrogen phosphate and modifiers such as ethyl acetate. Details of the assays of these analgesics in plasma are summarised in Table I. Minor modifications to the solvent composition were occasionally required for optimum separation of the drugs and metabolites from potentially interfering peaks in urine.

Using the ratios of the peak area or peak height of the drugs to those of internal standards, the calibration graphs were linear over a wide range of concentrations and passed through the origin. The limits of measurement are less than 1 μg ml^{-1} and the standard deviation of the methods over the therapeutic range of plasma concentrations is 3–5%.

TABLE I

DETAILS OF HPLC ASSAYS OF ASPIRIN AND METABOLITES, PARACETAMOL AND METABOLITES, DIFLUNISAL, ANTIPYRINE, PHENYLBUTAZONE AND MEFENAMIC ACID IN PLASMA

Drug	Metabolites	Plasma protein precipitant	Solvents*	Internal standard	Retention time/min†
Paracetamol	Sulphate, glucuronide, cysteine and mercapturic acid conjugates	Perchloric acid	0.1 M KH_2PO_4 - FA - IPA (100:0.1:1.7)	*N*-Propionyl-*p*-aminophenol	6.5
Aspirin	Salicyluric, gentisic, and salicylic acids	Perchloric acid	0.02 M KNO_3 in 2% AA - IPA - EA (100:12:4)	Acet-*p*-toluidide, benzoic acid	4.0
Diflunisal	—	Acetone	0.16 M KNO_3 in 2% AA - IPA - EA (55:25:20)	Flufenamic acid	4.2
Antipyrine	—	Perchloric acid	H_2O - IPA - TE (200:20:0.14)	Amidopyrine	3.5
Phenylbutazone	Oxyphenbutazone	Acetone	0.16 M KNO_3 in 2% AA - IPA - EA (55:25:20)	Flufenamic acid	6
Mefenamic acid	—	Acetone	0.08 M KNO_3 in 2% AA - IPA - EA (59:45:5)	Niflumic acid	6

* FA = 98% formic acid, AA = acetic acid, IPA = propan-2-ol, EA = ethyl acetate, TE = triethylamine.
† Primary drug.

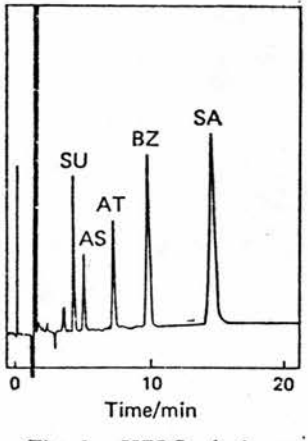

Fig. 1. HPLC of plasma from a patient with aspirin overdosage. SU = Salicyluric acid, AS = acetylsalicylic acid, AT = acet-*p*-toluidide, BZ = benzoic acid, SA = salicylic acid.

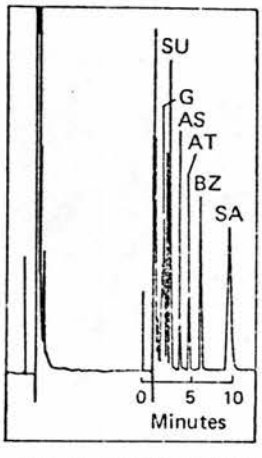

Fig. 2. HPLC of blank urine (left) and urine from a patient with aspirin overdosage (right). G = Gentisic acid, SU = salicyluric acid, AS = acetylsalicylic acid, AT = acet-*p*-toluidide internal standard, BZ = benzoic acid internal standard and SA = salicylic acid.

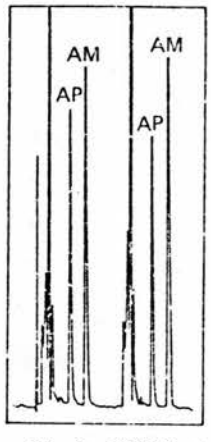

Fig. 3. HPLC of plasma from a patient who took 18 mg kg^{-1} of oral antipyrine (AP) for determination of the plasma antipyrine half-life. AM = Amidopyrine internal standard.

Details of the methods for the determination of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates have been published.[1,2] Chromatograms obtained during routine assays of aspirin, antipyrine and diflunisal in plasma are shown in Figs. 1–4.

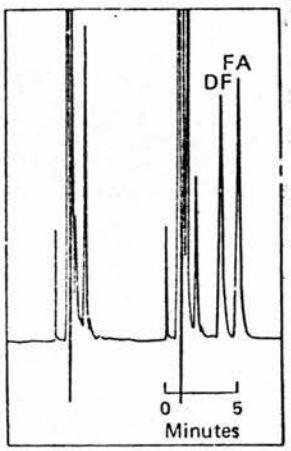

Fig. 4. HPLC of blank plasma (left) and plasma following a therapeutic dose of diflunisal (DF) (right). FA = Flufenamic acid internal standard.

These methods have been used for clinical pharmacological and pharmacokinetic studies in healthy subjects and hospital patients. No serious problems have been encountered with interference from endogenous compounds or other drugs. Extensive and detailed studies have been carried out on the metabolism of paracetamol following overdosage and the effects of treatment with sulphydryl compounds, such as *N*-acetylcysteine. In addition, the effects of different diets, other drugs, liver disease and gastrointestinal disease on paracetamol absorption and metabolism have been studied. This work simply could not have been done in the time without simple, rapid methods for the determination of paracetamol and its metabolites.

The methods could readily be scaled down for plasma samples of 100 μl and virtually all currently available, acidic, anti-inflammatory analgesics can be chromatographed under these conditions.

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Clinical Analysis of Steroids by HPLC

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Most of the physiologically active steroids possess some specific ultraviolet absorbance, *e.g.*, the Δ4-3-oxo configuration of corticosteroids at about 240 nm and the aromatic A-ring of oestrogens at about 280 nm, and can thus be detected directly in HPLC systems. However, the sensitivity is low and theory predicts that steroids measurable in plasma are at present limited to the most abundant corticosteroid, cortisol, its precursors if cortisol biosynthesis is blocked by disease or pharmacological agents, administered steroids and, during late pregnancy, oestriol and progesterone. In urine, most steroids have been reduced to non-

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FAILURE OF ALKALINE DIURESIS TO ENHANCE DIFLUNISAL ELIMINATION

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1 The effects of alkaline diuresis on the elimination of a single oral dose of 750 mg diflunisal were studied in six healthy male volunteers.

2 The plasma concentrations and half-life of diflunisal were not reduced by alkaline diuresis.

3 The 72 h urinary recovery of unchanged diflunisal was more than doubled with alkaline diuresis, but even so, only 5–7% of the administered dose was excreted unchanged.

4 With alkaline diuresis there was a significant increase in the mean renal clearance of diflunisal from 0.27 to 0.46 ml/min, but there was no significant correlation between the renal clearance of diflunisal and urine flow or pH.

5 However, there was a significant increase in the overall mean renal clearance of diflunisal from 0.22 ml/min over the period 0–24 h to 0.73 ml min from 48–72 h.

6 Forced alkaline diuresis is unlikely to be of value in diflunisal poisoning.

Introduction

Diflunisal (2′, 4′, difluoro-4-hydroxy-3-biphenyl-carboxylic acid) is a derivative of salicylic acid with similar pharmacology and toxicology. However, it is longer acting (Tempero, Cirillo & Steelman, 1977) and is claimed to cause less gastrointestinal bleeding (De Schepper & Tjanramaga, 1978) and to have less marked effects on platelet function (Smith Sibinga, 1977).

Diflunisal is a lipid-soluble, organic acid (pKa 3.3) and as such its renal clearance should be pH dependent (Milne, 1965). The effects of urine flow and pH on the renal clearance of diflunisal were studied by conventional clearance techniques in anaesthetized dogs (Baer, Breault & Russo, 1978). The net renal clearance of diflunisal was very low (about 1% of the glomerular filtration rate) and changes in the urine flow or pH had very little effect. About half of a single i.v. dose of diflunisal is excreted in the urine in dogs (50% unchanged and 50% as glucuronide conjugates) whereas in man, 95% of an oral dose appears in the urine, principally as glucuronide conjugates (Tocco, Breault, Zacchei, Steelman & Perrier, 1975). Forced alkaline diuresis has been recommended for the treatment of diflunisal overdosage, but the effects on its removal are unknown.

We have measured the plasma concentrations and renal excretion and clearance of the drug in the healthy male volunteers under different conditions of urine flow and pH.

Methods

Six healthy ambulant male volunteers aged 20–37 years weighing 60–82 kg were studied with informed consent. Each took 750 mg diflunisal (three Dolobid tablets) with 200 ml water following an overnight fast after which food, fluids and tobacco were withheld for 2 h. Venous blood was taken at 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48 and 72 h and urine was collected 2 hourly for 12 h and then 12 hourly for another 60 h. Urine volume and pH were measured and urine and plasma samples were stored frozen. Normal fluid intake, diet and activity were allowed, but alcohol and other drugs were not taken during the study.

The experiment was repeated in the same volunteers not less than 2 weeks later with administration of sodium bicarbonate capsules (3 g four times daily) the day before and for 48 h after the diflunisal. The fifth dose of sodium bicarbonate (the first dose on the second day) was given 1 h before the diflunisal. Fluid intake was increased to give a urine output of about 4 litres daily.

Unchanged diflunisal was estimated in plasma and urine by high performance liquid chromatography

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0306–5251/80/080163–03 $01.00

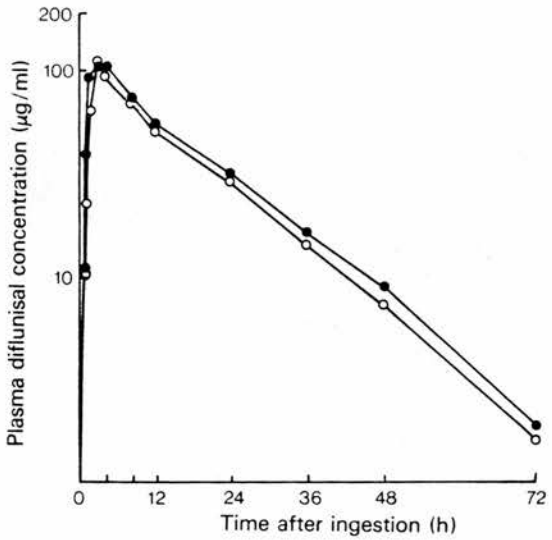

Figure 1 Mean plasma concentrations of diflunisal with (●) and without (○) alkaline diuresis in six healthy subjects after an oral dose of 750 mg.

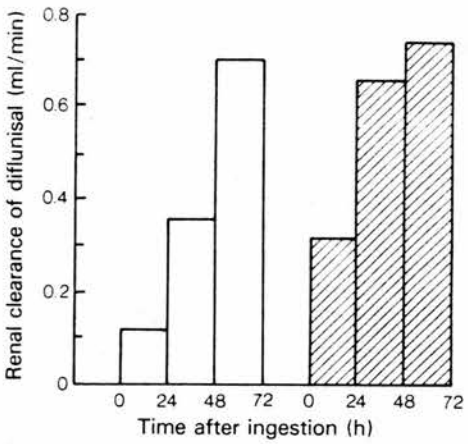

Figure 2 Increase in mean renal clearance of diflunisal with time following a single dose of 750 mg with (▨) and without (□) alkaline diuresis in six healthy subjects.

(Prescott, King, Brown, Balali & Adriaenssens, 1979). The plasma half-life, renal clearance and urinary recovery of the unchanged drug were calculated before and during alkaline diuresis. The plasma half-life was calculated during the linear elimination phase by the method of least squares and the area under the plasma concentration-time curve (AUC) by the trapezoidal rule. The renal clearance was calculated by dividing the amount of diflunisal excreted in the urine during each collection period by the corresponding AUC. Comparisons were performed using the paired, two tailed Student's *t*-test, with $P = 0.05$ as the minimum level of statistical significance.

Results

The plasma concentrations of diflunisal were similar throughout in both studies (Figure 1). Peak concentrations were reached within 3–4 h and the mean values were 112 ± 15 and 118 ± 11 (s.d.) μg/ml with and without sodium bicarbonate respectively (Table 1). The mean plasma half-life values were virtually identical in both studies (12.9 ± 1.5 and 12.5 ± 1.5 h). If anything, plasma concentrations were higher rather than lower during alkaline diuresis. the mean AUC up to 72 h was also greater with sodium bicarbonate, but the difference was not statistically significant (Table 1).

The 72 h urinary recovery of diflunisal was more than doubled by alkaline diuresis but even so, only 5–7% of the administered dose was excreted

Table 1 Effects of alkaline diuresis on the disposition of diflunisal

	Diflunisal	*Diflunisal with alkaline diuresis*	P *value*
Urine flow rate (ml/min)	0.9 ± 0.2	2.7 ± 0.2	< 0.005
Urine pH	6.3 ± 0.3	7.5 ± 0.4	< 0.001
Peak plasma concentration (μg/ml)	118 ± 11	112 ± 15	< 0.20
Plasma half-life (h)	12.9 ± 1.5	12.5 ± 1.5	< 0.60
Area under the plasma concentration-time curve 0–72 h. (μg ml^{-1} h)	1807 ± 207	1954 ± 343	< 0.30
72 h urinary recovery of diflunisal (mg)	22.4 ± 6.8	49.3 ± 13.2	< 0.005
Renal clearance of diflunisal (ml/min)	0.27 ± 0.25	0.46 ± 0.22	< 0.01

Values given are mean ± s.d.

unchanged. There was a significant increase with alkaline diuresis in the renal clearance of diflunisal from 0.27 to 0.46 ml/min (Table 1) but there was no statistically significant correlation overall between the renal clearance of diflunisal and urine flow or pH ($r = 0.08$ and $r = 0.04$ respectively).

Unexpectedly, there was a progressive and highly significant increase in the renal clearance of diflunisal with time (Figure 2) irrespective of urine flow or pH ($r = 0.75$, $P < 0.001$ with diflunisal alone and $r = 0.49$, $P < 0.001$ with alkaline diuresis). The overall mean renal clearance of diflunisal increased from 0.22 ml/min over the period 0–24 h to 0.73 ml/min from 48–72 h. There was a corresponding significant negative correlation between plasma concentrations and renal clearance of diflunisal with and without sodium bicarbonate ($r = ^{-}0.50$, $P < 0.001$ and $r = -0.66$, $P < 0.001$ respectively).

Discussion

Although the mean renal clearance and the 72 h urinary recovery of diflunisal were significantly increased by alkaline diureses, only 5–7% of the dose was excreted unchanged and there were no reductions in the plasma concentrations and half-lives. Plasma concentrations were actually higher with alkaline diuresis, possibly due to changes in absorption or distribution. The mean plasma half-life of 12–13 hours is consistent with the previous reports of dose-dependent elimination of diflunisal (Tocco *et al.*, 1975; Tempero, Cirillo & Steelman, 1978).

We were surprised to find no significant overall correlation between the renal clearance of diflunisal and urine flow or pH. On the other hand there was a highly significant relationship between the time after administration (and plasma concentrations) and the renal clearance of diflunisal irrespective of urine flow or pH. The explanation for these findings is unknown. Possibilities include induction of renal tubular transport of diflunisal with time, or that the maximum tubular secretory capacity was exceeded at higher plasma concentrations. Similar findings have been reported for the renal clearance of salicyl acyl glucuronide (but not salicylic acid) by Schachter & Manis (1958). We have confirmed that there is no correlation between the renal clearance of salicylic acid and plasma concentration and time after administration of therapeutic doses of aspirin in healthy volunteers. Interestingly, an opposite relationship has been described between the renal clearance of disopyramide and plasma concentrations. This was attributed to concentration-dependent plasma protein binding of the drug (Cunningham, Shen, Shudo & Azarnoff, 1977).

Forced alkaline diuresis has been recommended for the treatment of diflunisal overdosage (ABPI Data Sheet Compendium, 1979-1980). In one report it appeared to have little beneficial effect, although no measurements were made (Upadhyay & Gupta, 1978). The results of the present study provide no basis for the use of forced alkaline diuresis for diflunisal poisoning.

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